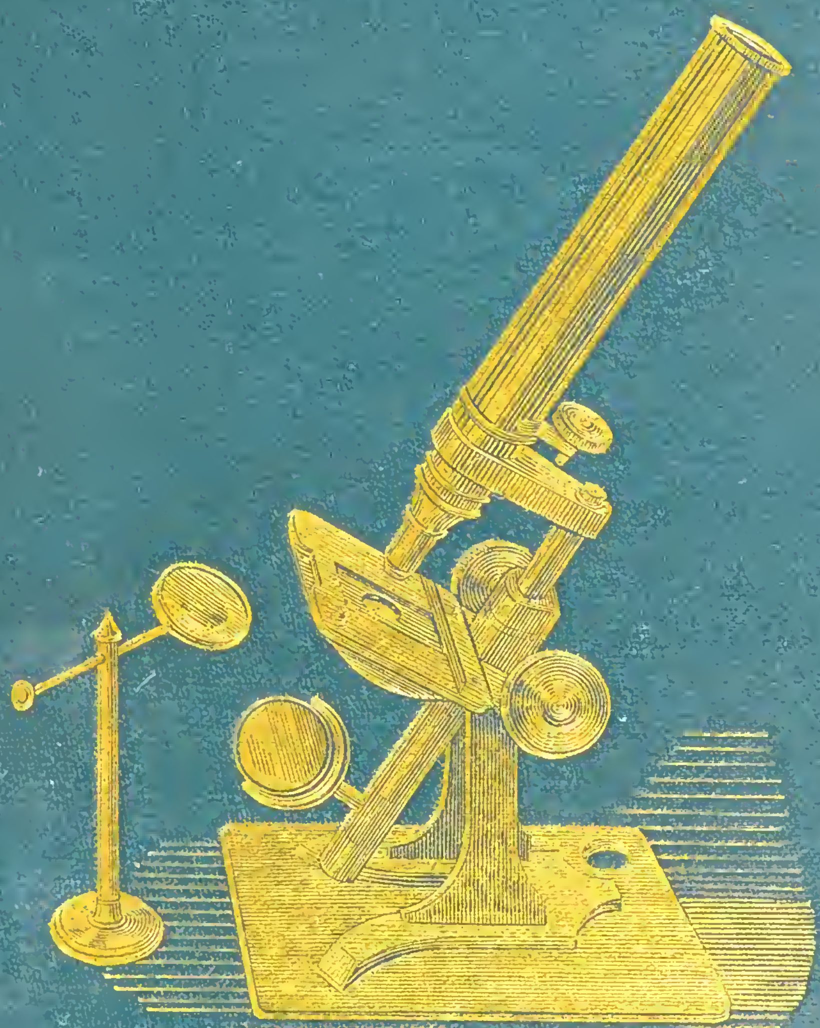
PHOTO-MICROGRAPHY

BY ANDREW PRINGLE.



LONDON:

ILIFFE AND SON, 3, ST. BRIDE STREET, LUDGATE CIRCUS, E.C.

WORKS: COVENTRY.

PRACTICAL PHOTO-MICROGRAPHY.

PRACTICAL PHOTO-MICROGRAPHY.

BY

ANDREW PRINGLE, F.R.M.S., Etc.

ILLUSTRATED.

LONDON:
ILIFFE & SON, 3, ST. BRIDE STREET, E.C.

CONTENTS.

PREFACE.

THE success following the author's former, larger, more pretentious, and more expensive treatise on Photo-micrography, and the desire to put into the hands of wholly inexperienced workers a manual, brief, explicit, practical, and moderate in price, have led to the production of this little book. This manual will be found to be purely practical; only so much of theory is given as seems necessary for the intelligent performance of the operations described and recommended. The text is, in fact, a mere description of operations daily performed by the writer. No apparatus is recommended on hearsay, nor is any statement made or step suggested outside the knowledge and practice of the writer.

A large number of scientific men, specially young medical men, are turning their attention to this branch of science; the writer's hope is that the instructions herein given may smooth the way to success, and lead the successful to still greater achievements in Photo-micrography than have yet been accomplished.

Thanks are due to many opticians, also to Dr. Sims Woodhead, and others, for the use of blocks for the diagrams; Messrs. C. Baker and Watson and Sons had blocks specially made for the writer's use.

No illustrations from the writer's negatives are given, partly because the utility of such is very doubtful, partly to obviate expense.

The Publishers are thanked for the handsome "get-up" of the book.

ANDREW PRINGLE.

BEXLEY HEATH,

November, 1893.

INTRODUCTION.

CHAPTER I.

Apartments and General Appliances.

AS the aim of this book is to give practical instruction, rather than theoretical or historical information, we shall not devote much space to preliminary remarks; but a few words on the more salient advantages of the science with which we have to deal may not be out of place. Photography may fitly be placed at the head of the graphic arts where record, demonstration, or education is the object in view; and photo-micrography is simply photography applied to the microscopic image. A drawing made by hand with the aid of a *camera lucida* or a "grapho-prism," however accurately it may be executed, cannot carry with it the conviction that is carried by a photograph; while the latter is produced with very much less expense of time and trouble, and can be reproduced in much greater numbers and at very much less cost. Moreover, photography gives us the power of showing our graphic production in the shape of a lantern slide on a screen to many observers at one time; and one of the processes on which stress shall be laid in this book is that of making lantern slides from photo-micrographic negatives.

There is, unfortunately, little doubt that pre-conceptions, "personal equation," and misinterpretation of microscopic images, tend to lead many of us astray in making drawings even with the optical appliances mentioned above; and while by faulty manipulation of the microscope we may be led into false photographic renderings, still the personal equation and prejudications are at least obviated by the use of photography. And almost all teachers and investigators are now daily acknowledging the use of photo-micrography; there are now few schools or institutions of science that do not use photography more or less for purposes of education and demonstration, and the number is daily becoming smaller. Photo-micrography is not only the graphic science of to-day, but will in even greater measure be the graphic science of the future.

Over and above all this there is the fact that those who have practised this science as a pastime, who have worked at it simply for its own sake, find this pursuit one of the most engrossing interest and fascination; and not without reason, for in our work we have much that is pleasing of optics, mechanics, chemistry and art, with, as a result, a lasting, useful, often beautiful, record of some of Nature's *arcana*. He who takes up photo-micrography seriously, and aims at the best results in the most advanced and difficult branches, must give much time and earnest attention to it; but he who merely wishes to follow a pleasant hobby may provide himself with apparatus less pretentious, and may take his pleasure less *au sérieux*.

At the outset it may be stated that this book is intended for those who are in earnest, and aim at pro-

ducing really good work, such as will bear comparison with any of the present day; and for this end two things are necessary—first, thoroughly good apparatus; second, careful study and pretty constant practice. The very finest appliances obtainable are not, after all, so very expensive, and in any case are in the end the cheapest; and intermittent enthusiasms will not conduce to equable results.

It is of great importance to have suitable apartments wherein to carry out the operations; and it will be well to provide separate rooms for the microscopic and photo-chemical parts of the work. Neither of these apartments needs to be large; and if we had only at disposal one medium-sized room we should put a partition so as to divide the room into two. Of course, the nearer the two rooms are to each other, the better; but the dampness usually found in the developing room would not be favourable to the microscopical apparatus. Again, a supply of running water, and a sink for waste, are of such import to the comfort, and even success, of the operator that we would make considerable sacrifice of other points to have these. As we deal with small plates only, a large sink is not required; a suitable one can be had of any dealer in photographic goods. Earthenware is usually recommended, but we prefer a wooden sink lined with medium-thick sheet lead, as less likely to get broken, and to break measures or dishes which might fall on it. The tap should have a "rose" at the delivery end, and in the sink should be a grating, or "hatch," whereon measures, dishes, etc., may drip.

All the appliances in the way of dishes, measures, and

the like, needed for quarter-plate negatives, or half-plate if the worker be ambitious, may be obtained for a few shillings.

The most important point to be considered in choosing the room for the microscopic part of the work is that of *freedom from tremor* under all conditions. A basement, or half-basement, apartment is suitable, provided we have means of keeping it dry. But an ordinary room with a good floor will answer well, especially if we do not propose to do very high power work. But if our work is, to a great extent, to be high power, then we shall do well to secure a thoroughly steady apartment from the first. And in such an apartment we must have a strong, solid, steady table to support our apparatus. If we are unable to secure a room above suspicion of tremor, we must fall back on a device for ensuring synchronous vibration of all the elements of our photo-micrographic apparatus. As will be seen, the various parts of our apparatus proper are mounted on one strong base, and we may place beneath such base cubes of gutta-percha, or even layers of carpet, so that any tremor may affect the entire apparatus simultaneously, the damage of tremor being thus nullified. We must have either synchronous vibration or none.

The illumination of what is often called the "dark" room is a matter of consequence, but a suitable lamp may be obtained from any photographic dealer. Ordinary light, as is well known, affects the sensitive salts in the photographic film, but if ordinary light be divided into its component parts by means of "spectrum analysis," it will be found that, though the whole of the visible spectrum

affects the sensitive salts, those rays which are nearest to the violet end of the spectrum affect the plate much more than do the rays more nearly approaching the red end of the spectrum. In fact, it may be taken that in practice the red and orange parts of the spectrum do not affect an ordinary plate to any appreciable extent, unless the plate is exposed to such rays for a very long time--for a longer time than is usually required for the "development" of the plate. Consequently we are able to watch the progress of development by using red or orange, or even yellow, light in the room where the operation is carried on. But if the light from the lamp, or from the sun, is allowed to reach the plate without the chemically active rays having been stopped, the plate will certainly suffer. Accordingly we use, to filter out the "actinic," or chemically active, rays, some medium, such as transparent ruby glass, or translucent, green-yellow paper, or other fabric. Workers who use ordinary plates generally prefer the yellow fabrics, but for our work it will be necessary to use ruby-coloured material of some sort. The reason is this: all the best photo-micrographers have found that it is a great advantage to use "orthochromatic" or "colour-correct" plates. Now, by a process to be described later, plates are rendered more than normally sensitive to the yellow and orange rays of the spectrum, and relatively less sensitive than usual to the violet and blue rays; so that a yellow light, or even an orange light, in the developing room, would not be "safe;" or, in other words, would affect the sensitive salts in the orthochromatic film. So the photo-micrographer ought

to provide himself from the start with a ruby lamp; a yellow light will answer when orthochromatic plates are not in use, as for the operations of bromide printing and slide making. Lamps for the dark room can be obtained with both yellow and ruby glasses or fabrics.

The lamp should be large, and, if permanently fixed, should be arranged so that the heated air and the products of combustion are carried out of the room. If gas is used in the lamp, the tap for turning the gas up and down should be on the outside of the lamp, and a "regulator" burner is of great value, as giving at any time a standard light whereby the negatives may be examined during progress of development. The lamp should be at one side of the operator, or should, in any case, be arranged so that its light shall not shine into the eyes of the worker. Artificial light, on account of its equability, is preferable to daylight, which varies so much in strength and quality.

To test the "safety" of a lamp, lay a plate of the kind to be used in the place where development is to be performed, covering half of the plate with an opaque material; leave the plate so for about five minutes, the lamp, of course, being lighted, and then develop with one of the developing solutions. If both halves of the plate remain clear the light is safe; but however safe the light, the plate should never be exposed to the light unnecessarily. During development it is well to cover the dish containing the plate with another dish or a piece of cardboard.

A so-called "Matchless burner" is a great convenience in the operating room; this is a device whereby an ordinary gas burner can be turned very low, so as not to affect a

plate, without being turned out; the light can be turned up without loss of time, or relighting.

All the appliances of a general nature required for the photographic part of our work are kept in stock by all dealers; and these appliances are, happily, neither numerous nor expensive.

In the operating room there should be a set place for everything used; confusion and dust should have no place in the room. Care must in particular be taken that the "hypo" solution be not splashed about the floor or the table. Every dish and measure should be cleaned immediately after use; this will be found economy, not waste, of time.

CHAPTER II.

The Microscope and its Parts.

EVERY microscope intended for serious use must have certain qualities if satisfactory performance is to be expected. The fitting of every part must be of the most accurate and lasting workmanship; its general design must be convenient and handy; and it must be more or less "solid" and entirely steady. These qualities are required for photo-micrography in a special degree, for the test of projecting the image on a plate many inches distant from the ocular, and of keeping it absolutely steady there for many seconds or even minutes, is much more severe than that of merely projecting an image on the retina, the hand being on the fine adjustment all the time to correct any alteration that may take place in the relation between the various parts. Consequently the greatest care should be taken in selecting a "stand" for our work; one that may answer fairly well for ordinary observation may utterly break down under the test of photography. Let the conveniences of the pattern be ever so great, let the instrument have every known "movement," all is of no value if the instrument is not absolutely steady, rigid in every part, and accurate in every fitting.

Leaving aside accuracy of fittings as an attribute of every good stand, let us consider the matter of steadiness. The two leading types of microscopes, so far as the base

is concerned, are those on the "horse-shoe" foot, and those on a tripod foot. Stands on the former base are moderately steady when the optic tube is vertical, but even in this position they are not difficult to overset. When the tube is put to the horizontal position they are very far from steady; the least push will overturn them, and the least tremor affects them seriously. The horse-shoe base is not the base for photo-micrography, and we cannot but wonder at some of the first Continental makers persisting in making this base alone, and still more at their recommending it for this purpose. Infinitely superior for our purpose, to say nothing of general considerations, is the tripod base, the tripod having as wide a "straddle" as convenient.

Again, it will be seen, on examining most of the Continental models, that the optic tube is supported and works on a very short body or "limb." Some of the best-known models have this support only about three inches long. The body on which the tube, actuated by the coarse adjustment, moves, should, for the sake of steadiness, be as long as possible. Let the reader compare the short limb of a Zeiss stand for photo-micrography with that of the stand figured No. 1. The calibre of the tube is often too small; it should not be less than 1¼ inch, especially for work without the ocular. Still again it may be observed that many good stands, when set at the horizontal, have so much in front of the stage, or of the pivot on which they turn (in the Continental models this pivot is actually below the stage), that the weight in front is greatly in excess of that behind, another most dangerous

feature as regards instability. Such stands in this position, which is in nine cases out of ten the position for our work,

Fig. 1.

look grossly ill-balanced, and *are* no less so than they seem. Very fine work has been done with stands such as

these, but this has been due to the skill and patience of the workers rather than to the inherent excellence of the instruments. We have heard a rumour, and we hail it with pleasure, that the firm of Zeiss at least proposes to make and supply a stand better designed in these respects than their present pattern.

Even when we have a proper base and a long body, there is evidently at least a theoretical danger of tremor at the ocular end of the tube, and to meet this Messrs. Swift and Son made, on lines suggested by the writer, the instrument fig. 7. Here it will be noticed that not only is the body very long and solid, but the ocular end is supported by a trestle, the result being perhaps the most steady instrument ever produced. Mr. C. Baker carried out for us the same principle in the instrument fig. 8, which is the one we use regularly in our own practice. In these cases it is to be observed that the trestle is attached to the body-support of the tube, and the support must be fairly long if the trestle-support is to give the full advantage. If the support of the tube were short, the trestle would require to be so far forward that it would lose most of its value as a "steadier."

At one time the common habit was to cause the fine adjustment rack to act on a nose-piece fitting inside the optic tube, but of late years the tendency has been to cause this adjustment to actuate the entire tube. Now evidently it is a dangerous principle to place on the most delicate machinery of the whole instrument this considerable weight; the result would seem to be certain damage to the adjustment in a short time. According to the

writer's experience, many instruments of Continental make, fitted with this kind of fine adjustment, have given way under the strain, and are valueless as instruments of precision. Further, the instrument usually acknowledged to be the best that is made, viz., that of Messrs. Powell and Lealand, has the nose-piece arrangement. But it must be added that at least two instruments, with fine adjustments actuating the whole tube, have stood the test of hard work and time in the writer's hands, viz., the "Campbell differential screw" in Mr. Baker's "Nelson" stand, and the modified Zentmayer lever in the "Edinburgh" stand, of Messrs. Watson & Son. There is also the "side lever" arrangement of Messrs. Swift, of which we have good accounts from experts, but no experience of our own. For use with the higher power "apochromatic" objectives of Zeiss, which are probably the best objectives to be obtained for our work, the nose-piece fine adjustment arrangement has the serious, if not fatal, fault that in use it alters the length of the tube; and the "correction" for these objectives depends on, and is regulated by, this length. At the same time, it must be admitted that the variation in length produced by the working of the fine adjustment is very small, and, except in the most critical work, may be overlooked. For general microscopy we might prefer the nose-piece style of adjustment, but in photo-micrography all our experience leads us to prefer the entire tube arrangement. Whichever of the systems we choose, great care should be taken to make sure that the fine adjustment works straight, without the least side motion in its course; that it "loses no time," *i.e.*,

that it answers to the least touch on the screw by which it is manipulated; that its motion is sufficiently slow, and that it is absolutely free from a tendency to "jump" forward or backward. The coarse adjustment is usually worked by a rack and pinion; but there is a vast practical difference between a good rack and a poor one. The rackwork should be diagonal and not perpendicular to the axis of the tube (fig. 2). This diagonal

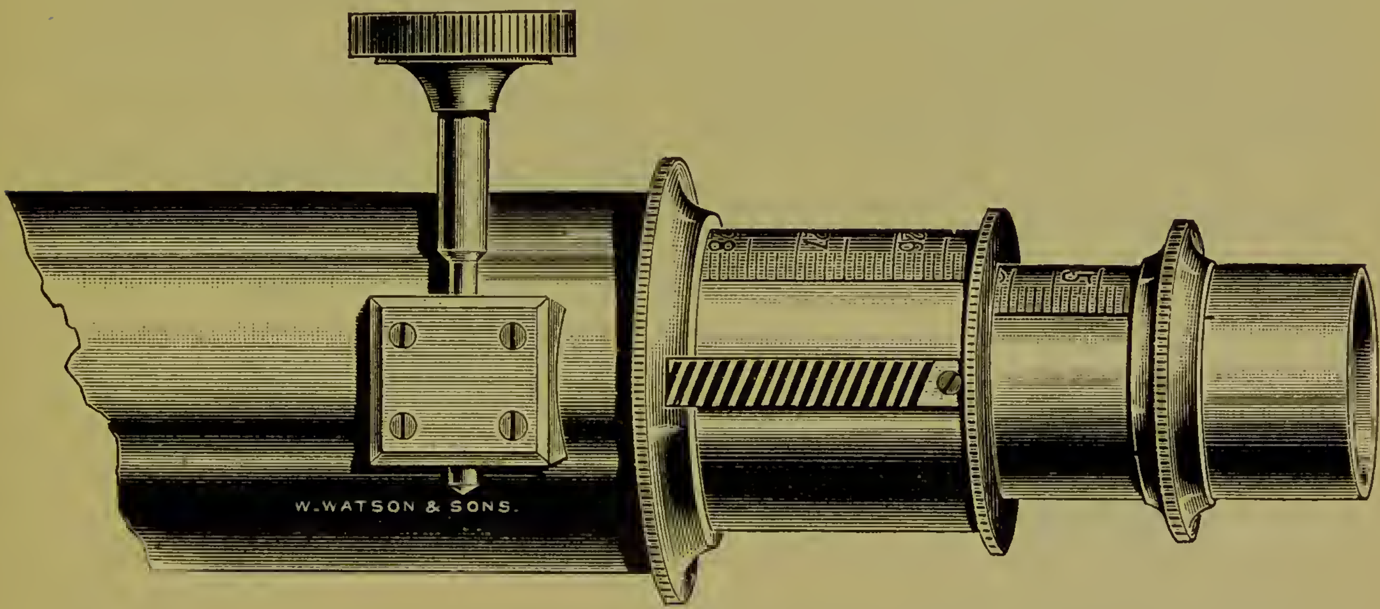


FIG. 2.

arrangement is found in almost all of the best stands of the present day. As pointed out in Dr. Dallinger's edition of "Carpenter," the groove, of whatever shape, in which the bar of the coarse adjustment works, should be *cut and sprung*, so that when the fittings work loose, as in time they surely will, they can be tightened up by screws. Except a bad fine adjustment nothing is so exasperating as a bad coarse one; a good coarse adjustment, on the other hand, is very great boon; we have

two microscopes with which we can work comfortably, using an "eighth" and the coarse movement alone; one is a new Student stand by Watson, the other is a Powell and Lealand, dated 1866.

The "stage" next requires consideration. The hole in it should be of ample size; a clear inch of diameter is not enough, and the stage diaphragms, and even "iris" arrangements, sometimes seen in the stage, serve no purpose that we can discover. A "mechanical stage" is, without doubt, a very great convenience, and all first-class stands are fitted with one; but it adds to the cost considerably, and it certainly can be dispensed with. The photo-micrographer will find a good mechanical stage a great boon, chiefly for two reasons. First, by means of it he can search the whole of a preparation without fear of missing any part, which cannot be said for the manual practice of searching for, say, bacteria with a high power. And second, it is easy to mark a spot once found by either of two devices. These stages are usually marked with two sets of minute divisions at right angles to each other, so that a spot once found, the "reading" of these marks may be noted, and the same spot found at any future time by simply setting the stage to these readings. Or a "Maltwood" finder may be used for the same purpose. This finder consists of a photograph, small in size, of a number of squares figured consecutively in order, two numbers in each square; and it is used thus: the worker, coming on a spot which he wishes to register, removes the preparation and substitutes for it the finder, noting what square is in the field.

When, at any future time, he wishes to find the same spot, he places the finder on the stage, finds the square previously noted, removes the finder, and replaces it by the preparation. In both cases, for marking by the figures on the stage, and for finding by the finder, there is on the stage a *stop*, against which the preparation and the finder are placed. Few of our leading pathologists or bacteriologists use a mechanical stage, and the trouble to those who have to photograph their preparations is often immense

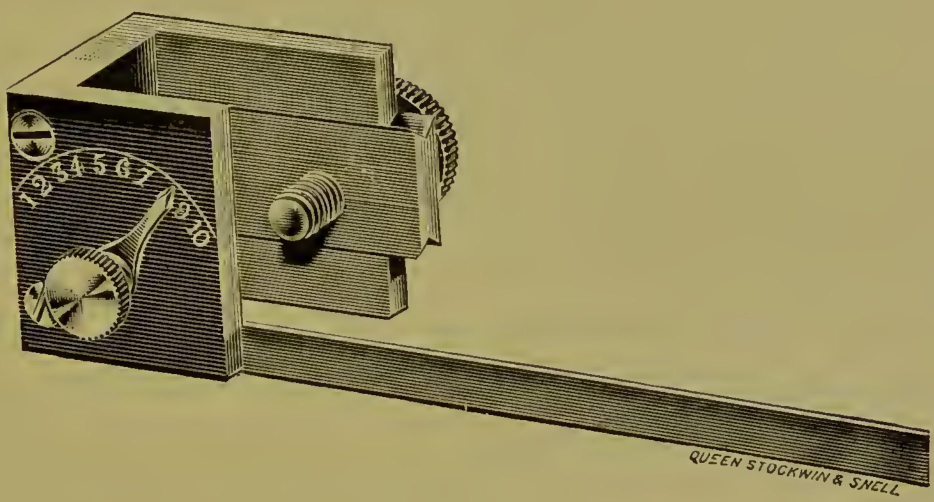


FIG. 3.

—as when some particular organism is to found—and our friend, Mr. E. C. Bousfield, has designed an extremely useful little apparatus for "Maltwooding" on a plain stage. It is figured here, No. 3, and is made by Mr. C. Baker.

"When the desired field is seen, the slide is firmly fixed by the clips; the long thin bar of the apparatus is then inserted beneath the clips, and the rectangular elbow formed by the junction of this bar with the body is closely applied to the slide. This being made sure of, the screw

beneath is tightened so that the frame is firmly held in position. The slide is now removed and the finder substituted for it, and the number read off," etc. "When it is desired to refind the field the apparatus is placed upon the stage with the finder in its angle, the required number found, the frame fixed in position, and the slide substituted for the finder."

The fitting of the mechanical stage should be as accurate as any part of the stand, otherwise great vexation will arise. Nothing is more annoying than a stage so badly made that the object keeps moving out of sight, or out of focus, at every movement of the mechanical stage. The slide, *i.e.*, the preparation, should never be clipped *down to* the stage-plate by springs as is so common—at all events the clips should be quite weak, just strong enough to keep the slide in place; the proper clips act on the edges or ends of the slip. We do not know of any mechanical stage so good in all respects as that of Powell and Lealand, and in pointing out some of its excellencies we shall be stating the *desiderata* for such a stage. The motions in both directions are effected by racks and pinions, and as these are worked the milled heads do not move away from the hand. The stage-plates move on properly-shaped grooves, the fittings being *sprung* as described under the head of the coarse adjustment bar. The milled-head screws are placed close together, and in such position that we can effect vertical or lateral movement without moving the hand from one place; and the pinion of one "motion" is carried through to the left side of the stage, an arrangement which is of great convenience sometimes.

Below the stage we have the *substage*, which is intended to carry the substage condenser, and occasionally other appliances connected with illumination of the object. Except for the very simplest and lowest power work a condenser may be taken as necessary; and a condenser without a good focussing and centring substage to carry it is almost useless. The remarks made as to the necessity for a good coarse adjustment hold equally true for the substage; and in the highest class stands we have a fine adjustment also to the substage. A "swinging" substage is seldom used nowadays, but a centring arrangement there must be for the substage. So much attention has of late been given to the use of the condenser that the substage has also been much studied, and most stands of any pretension have a good substage. A mirror usually forms part of a stand as sold, and in certain circumstances, as when we are working with the stage horizontal, the mirror is of importance. This matter will be discussed later.

The beginner must examine the optic tube of his instrument, and make sure that the interior has no shiny spots or areas; the inside of the tube should either be thoroughly coated with a dead black, or lined with black velvet. Any shiny parts will lead to trouble in some of our after performances.

En résumé: A stand for photo-micrography must be absolutely rigid and steady; its fittings and "motions" must be of the best workmanship; it must be capable of being used in the horizontal position; it must have a good substage, focussing, and centring; it may with advantage have a mechanical stage, a good one, if any; and its tube must be dead black inside.

The length of the tube is a matter on which opinions differ; Continental workers generally use a tube of about 6½ inches, the English size being about 10 inches—160 and 250 millimètres respectively. Some makers think to accommodate all clients by making a short tube and putting to it a considerable length of "draw"; but this, sooner or later, leads to trouble, for the draw-tubes work loose in time. But one length of draw is not only permissible, but, under certain circumstances, necessary, as where we use the higher power apochromatic objectives. Consequently we recommend one draw, and this is most conveniently worked by a rack and pinion, as seen in our fig. 1. Objectives are usually found to work best at about one or other of the standard lengths named, and the apochromatics are made deliberately for a short or for a long tube, and do not work well except at about the tube length for which they are intended. On the question—which is preferable, the short or the long tube? the writer has no opinion to give. The one is as good as the other, only we find, and others find, that a long tube apochromatic works fairly on a short tube, but, as a rule, a short tube one does not perform well on a long tube. A tube may safely be made adaptable from about 6 to 8 inches, or from about 9 to 11 inches; any greater draw is apt to be weak.

The stands chiefly intended and, in our opinion, best adapted for photo-micrography are those of Baker (Nelson model), Watson (tripod foot), Swift (Jackson—Lister, or Wales pattern), but, keeping in mind the remarks made in this chapter, the beginner need not fear to employ any really steady, well-made stand.

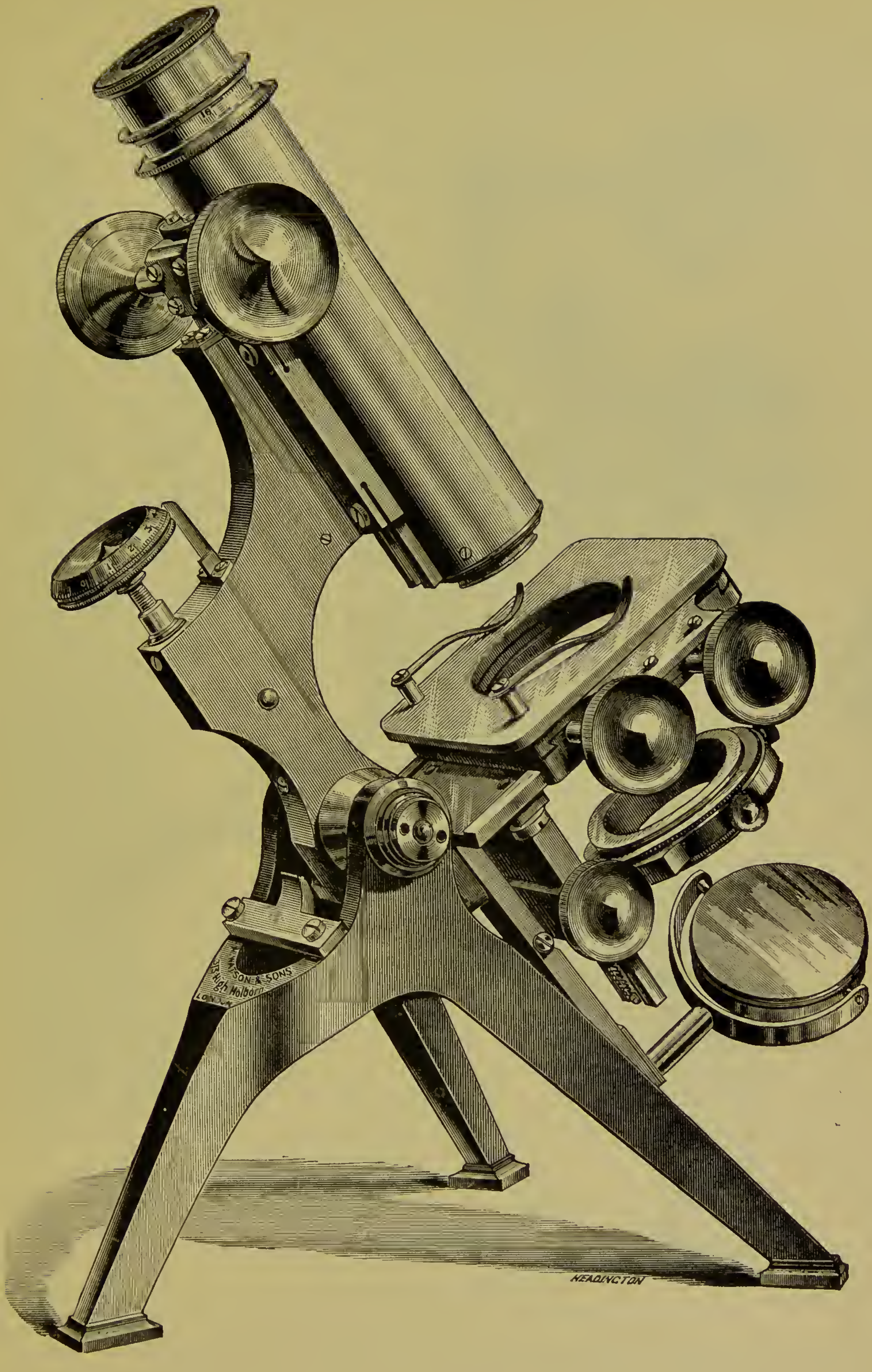


Fig. 4

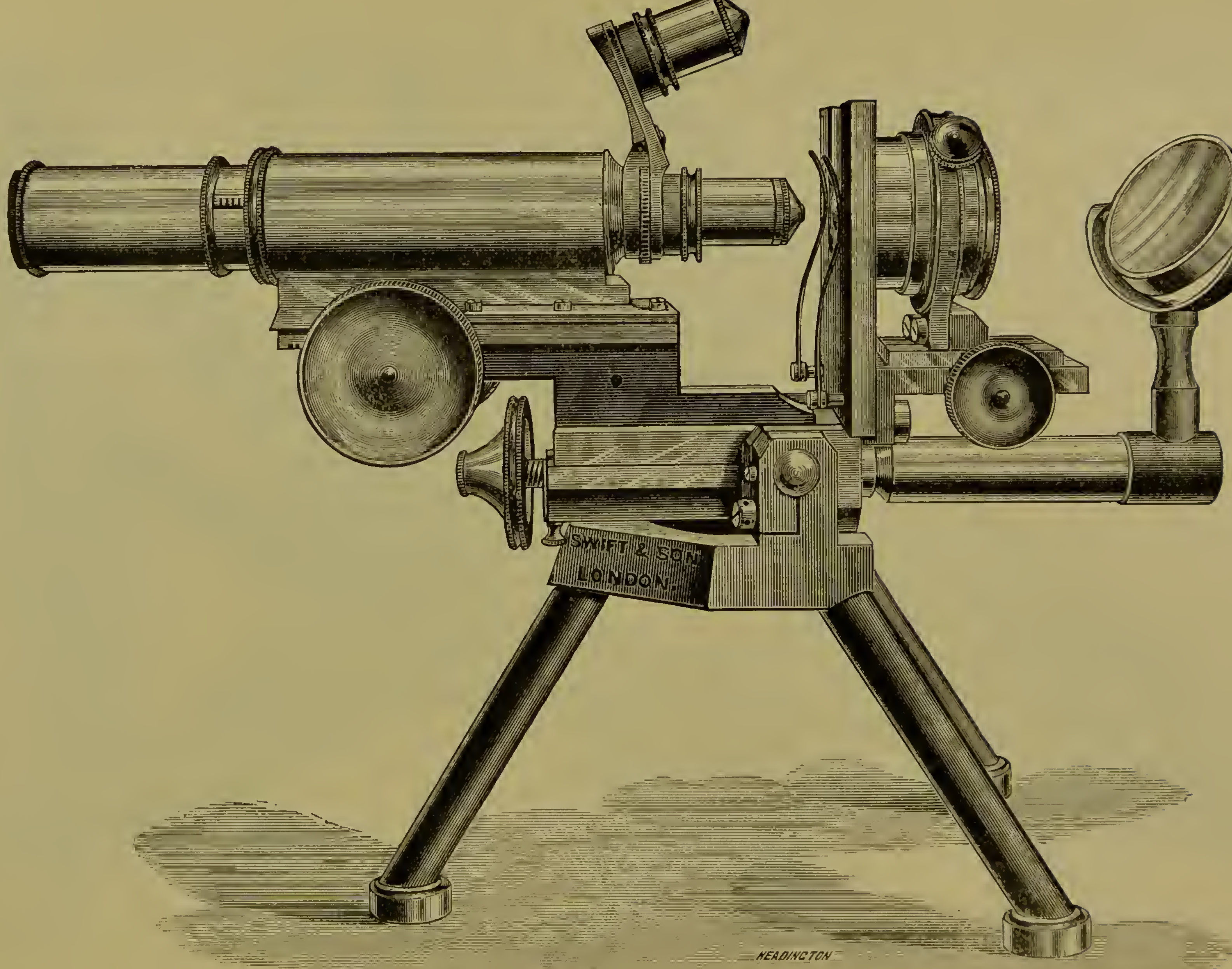


Fig. 5a.

Fig. 4 shows a very good stand by Watson. It is a "Student's" stand of the highest class, and, considering its design and fittings, is far from dear.

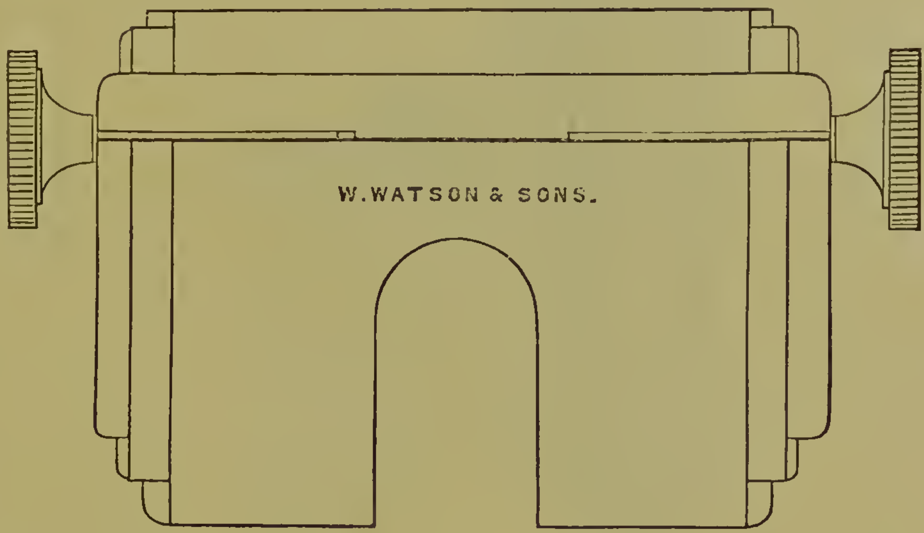


FIG. 5.

Fig. 5 shows the stage of another good stand of the same firm. This, from its abnormally large stage, is specially adapted for examination and photography of "plate-cultures," and the like.

Fig. 5A shows a new stand by Swift, specially well adapted for photo-micrography; it is called the "Histological" stand. We choose the horizontal position to show the evident stability of the instrument in the position required for most of our work.

Fig. 5B is a cheaper stand by Beck, also suitable.

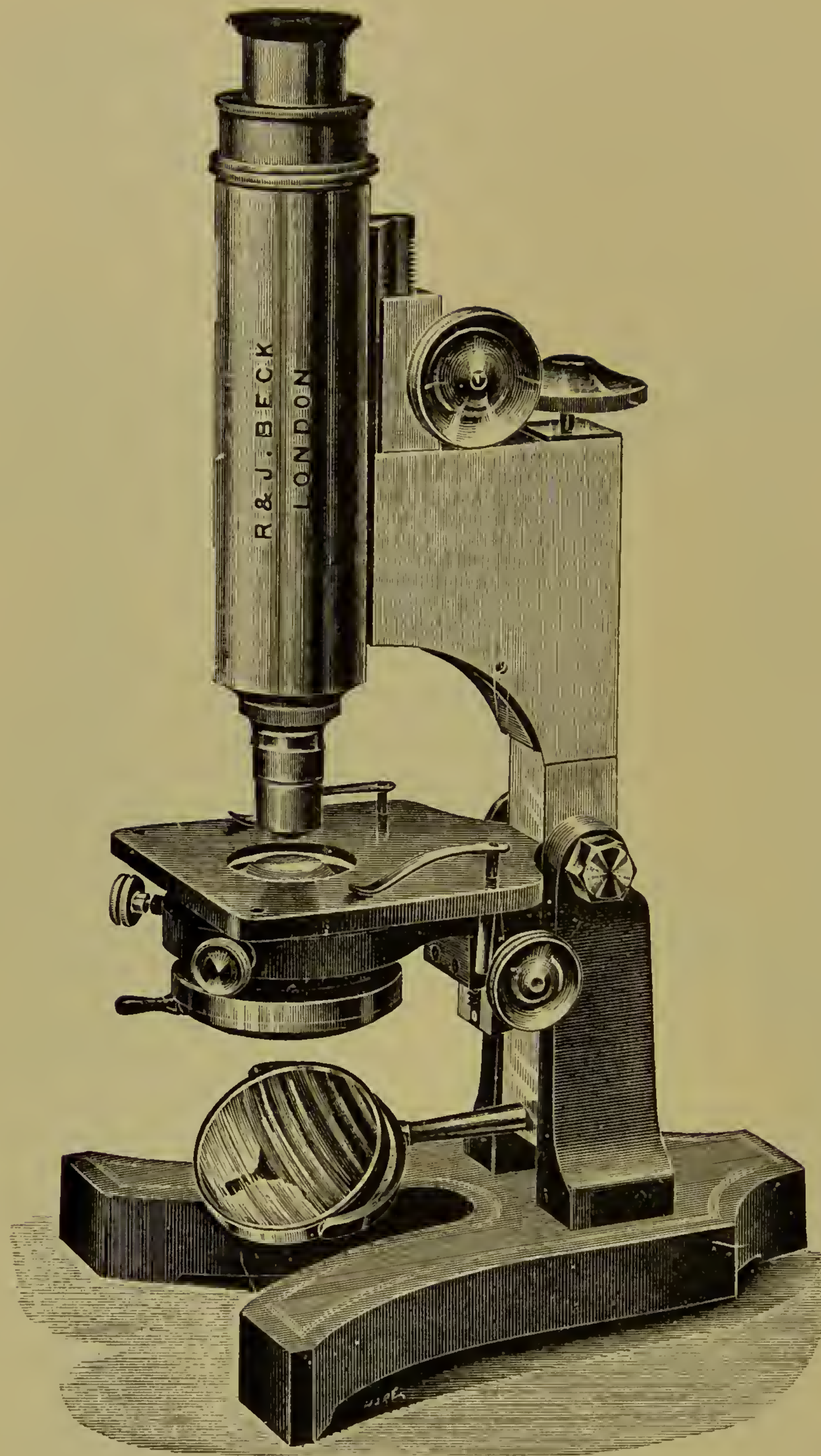
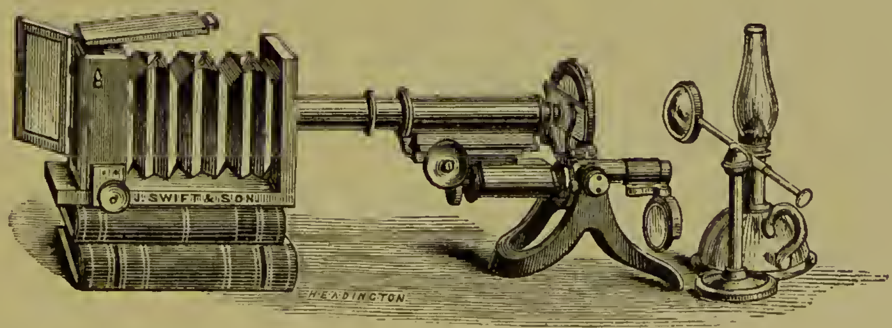


FIG. 5B.

CHAPTER III.

Photo-micrographic Apparatus. (1.) Horizontal. (2.) Vertical.

OUR complete apparatus consists essentially of a camera, a microscope, and appliances for illumination. Though not absolutely essential, it is highly important that all these parts should be fixed to one base. We view with distrust any apparatus where, for instance, the camera is supported by one table or stand, and the microscope or source of light by another; any such arrangement savours of unsteadiness, and if there is tremor, the parts will probably not vibrate synchronously. We have seen fairly good work, at moderate magnification, done with an "apparatus" put together somewhat after the fashion here shown; but anyone



proposing to occupy himself with even low power simple work will save much worry, and much failure, by providing himself with an apparatus made for the purpose, however simple and unpretentious. Anyone possessing a

suitable microscope, a camera of fairly long stretch, and a lamp, may, with a little ingenuity, and a little joiner-work, construct a very good apparatus for himself. But as almost all opticians now keep in stock suitable apparatus, at very reasonable prices, it is probably the safest, and possibly the cheapest, plan to obtain one of these apparatus.

The leading point of such an apparatus is that the light, the axis of all the optical parts, and the centre of the plate, shall be, in the "normal" position, in one line; the plate and the stage parallel to each other, and at right angles to the optical axis of the system. The stage, or the preparation on it, the condenser, the bull's-eye, and the radiant have a certain amount of possible movements, vertical and horizontal, and the plate may also be movable in a direction vertical to the optical axis, but at all times the plate must be parallel to the stage; and the optical axes of the objective, the tube, and the ocular remain fixed at all times.

The camera should be capable of being stretched to the length, from front to back, of at least 30 inches; if much work is to be done without the ocular the stretch may extend to 60 inches. Though, as a rule, the negatives made are not larger than a circle of 3in. diameter, still the size of camera usually supplied for the work is "half-plate," 6½ by 4¾ inches. This larger size is occasionally desired, but in our practice very seldom, and it is always easy to produce, or to get produced, an "enlargement" from the smaller size to the larger without any serious loss of quality. When the camera is of half-plate size, the usual

plan is to have in the "dark slide" a "carrier," by which we use in our larger slide a smaller plate, such as a "quarter-plate," 4¼ by 3¼ inches, or, as some prefer, 3¼ inches square. The latter size takes all that we usually require, but does not allow so much room for writing on the negative as does the full quarter-plate size The dark slide must not slide into the camera by long grooves, but by a "bayonet" joint, and it should go into position very easily, otherwise the focus may be disturbed at the critical moment.

Cameras with such a stretch as 30 inches are generally made with bellows for the body, and this is the best system; the bellows need not be of fine leather, "cloth" bellows answer quite well. The bellows should be supported in some way about the middle, so that they may not "sag" and interfere with the projected image when a long camera is being used. At the front of the camera, just behind the end of the microscope tube, there should be a shutter inside the camera, working easily, and operated from the outside by a milled head screw, or other handy device. This is for starting and closing the exposure. The back of the camera may have a rise and fall, and a side motion, so that the image may at any time be made to fall exactly on the centre of the plate, but this is rather a luxury than a necessity. The front of the camera is fitted with a short length of metal tube which fits loosely into a cap fitted to the ocular end of the tube; this is to prevent light from outside from reaching the sensitive plate during exposure. The inside of the camera must be dead black all over; and close to the

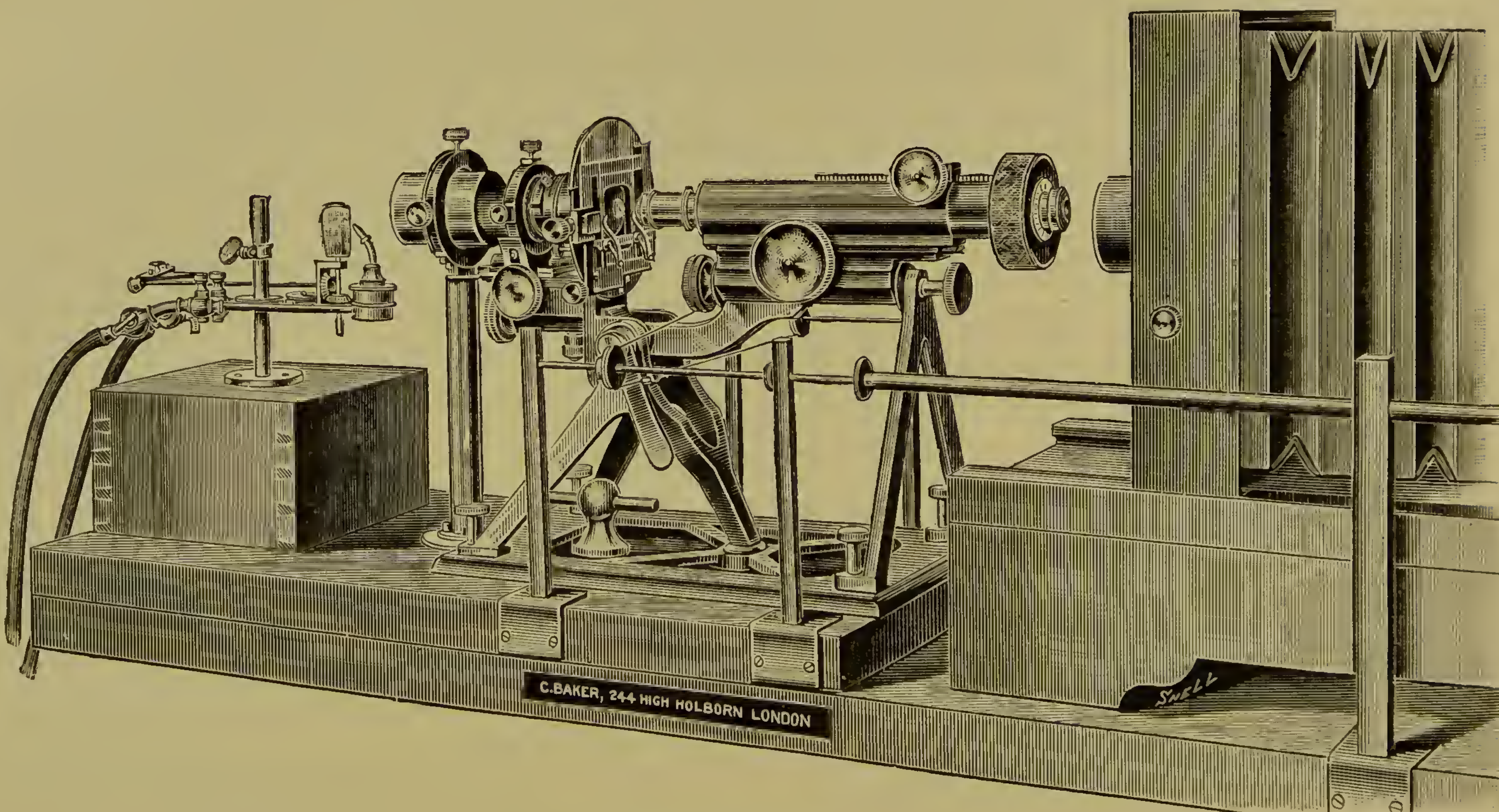


FIG. 6

plate may be a sheet of metal or card having a circular aperture of the size desired for the negative, 2¾ or 3 inches diameter.

When the camera is stretched to, say, 30 inches, it is evident that we cannot examine the image on the ground glass and manipulate the adjustments of the microscope at the same time. We require, therefore, some arrangement enabling us to reach and operate the fine adjustment from the rear part of the apparatus. A "Hooke's joint" is used by some, and has been used by the writer, but it is far from a satisfactory solution of the problem, for when, after focussing, it is laid down, there is great danger of the focus being shifted. Much better is a rigid rod extending to the back of the camera and actuating the fine-adjustment-milled-head by means of a cord passed round rod and milled head. In the instruments of some workers the cord passes round a pulley-block on the rod, then round the milled head, then round another pulley on the side opposite to the rod, then back to and round the rod. This arrangement is seen on our own instrument, fig. 6. The alternative method—and it is perhaps the better—is to counterpoise the power on the rod by means of a weight attached to the cord after it has passed over the pulley opposite to the rod. Unless, however, the apparatus stands on a narrow bench, so as to allow the weight to hang down some distance below the stand, the available length for the motion is somewhat restricted, and may lead to inconvenience. This defect may be obviated by boring a hole in the table, and letting the cord pass through this hole. But we have never had any trouble

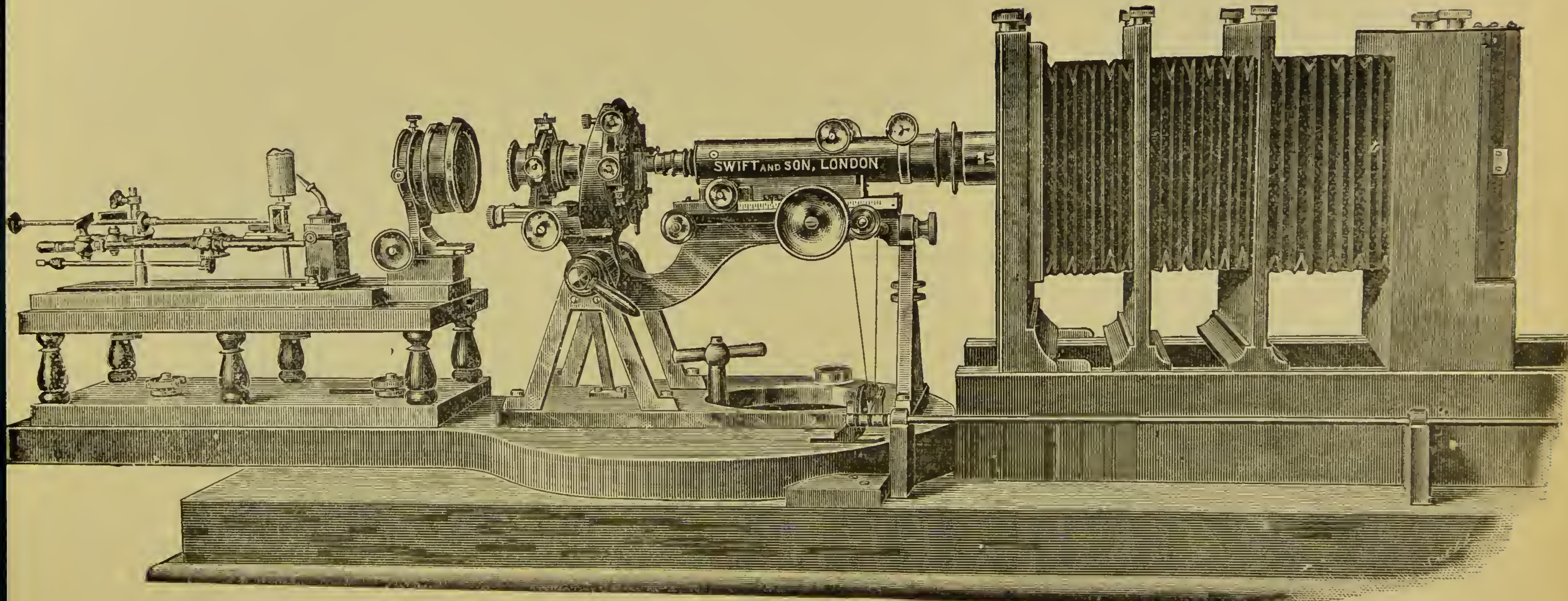


FIG. 7.

with our own arrangement, wherein the cord passes round the whole circuit. Any arrangement—and there are some—where the cord passes simply round rod and milled head is to be avoided ; the microscope is sure to be more or less pulled aside by such an arrangement when the rod is actuated by the hand.

Fig. 7 shows what is probably the most elaborate apparatus ever constructed for photo-micrography. It was made mainly to our design, for the Royal Veterinary College, by Messrs. Swift and Son, and whatever merit there may be in the design was admirably carried into effect by the notable excellence of the workmanship ; moreover, the details are, to a great extent, due to the experience and ingenuity of Messrs. Swift. The trestle already mentioned, as supporting the ocular end of the tube, will be noticed, and the other characteristics of strength, and of length of body, are seen at a glance. The arrangement for effecting the fine adjustment motion is intricate, and as it was, later, altered, it need not be described here. A large T-headed screw, under the body, however, requires notice, as it is an essential part of an arrangement which we put forward as important for convenience of working under ordinary conditions, though we do not say it is a necessity, and though it is not used by some of our best workers. The microscope and the illuminating apparatus are mounted on a turntable, which revolves on the pivot formed by the large screw under discussion ; so that the operator may stand or sit at one side of the entire apparatus, turn the microscope and light out from the central line, apply his eye directly to the ocular, and

arrange the lighting, centring, tube or collar-correction, etc., and then return the microscope and light to the normal or axial position. This is infinitely easier and handier than putting the head in between the microscope and the front of the camera, not to mention arranging the object on the microscope wholly detached from the apparatus, and then setting the microscope in position. The back of this camera is fitted with sliding parts so that the slide carrying the sensitive plate can be raised or lowered, and pushed to one side or the other, and this device is to be found in later, if not earlier, apparatus. We do not lay much stress on the advantage of this arrangement, *if the microscope is permanently fixed on the apparatus*; but if the same microscope is used for general observation and for occasional photography, then we recommend the rise-and-fall, and the traversing motions, for it is impossible to set down the microscope exactly at the same place always, and the slightest deviation from the normal position of the microscope will throw the image on the plate considerably out of its normal position. The apparatus now under discussion is expensive, but will be found to fulfil every desire of the most exacting worker, especially with a modification of the focussing device, for which the counterpoise system may be recommended.

We next give a figure and brief description of the instrument which we use for own work, and it is the outcome of some years of experiment and frequent modification. This instrument (figs. 6, 8 and 9) was made for the writer by Mr. C. Baker, of High Holborn, and it leaves little or nothing to be desired in workmanship,

besides being produced at a really moderate price. (See Appendix.)

Here, again (fig. 6), is seen the support for the ocular end of the tube; the body bearing the tube is long and strong; there is a rack and pinion for altering the length of the tube, which is at its normal a 10 inch one; we have the turntable device for the microscope and light; a good mechanical stage; a substage with both coarse and fine focussing adjustments, and with centring as a matter of course. The thinner part of the focussing-rod bearing the pulley fits into the thicker part by a square fitting; these parts are severed when the turntable is turned away from the axial line. The ocular end of the tube and the front of the camera are each fitted with a cap; when the image is projected on to the focussing screen of the camera, these caps are slipped one into the other to prevent stray light from impinging on the sensitive plate. The base of the whole is a thick plank of teak, and all the parts are strongly clamped to this base. The microscope is first firmly clamped to a metal platform, from which it can be removed at will; but ours is never removed except for "alteration or repair." One figure (8) shows the instrument in position for ocular examination of the object down the tube; the other (fig. 9) shows it ready for an exposure to be made. The round finger-piece of the flap shutter inside the camera can also be seen on the right near the front of the camera.

The ground glass found in an ordinary photographic camera will not answer for focussing the image of microscopic objects projected upon it through an objective. We use

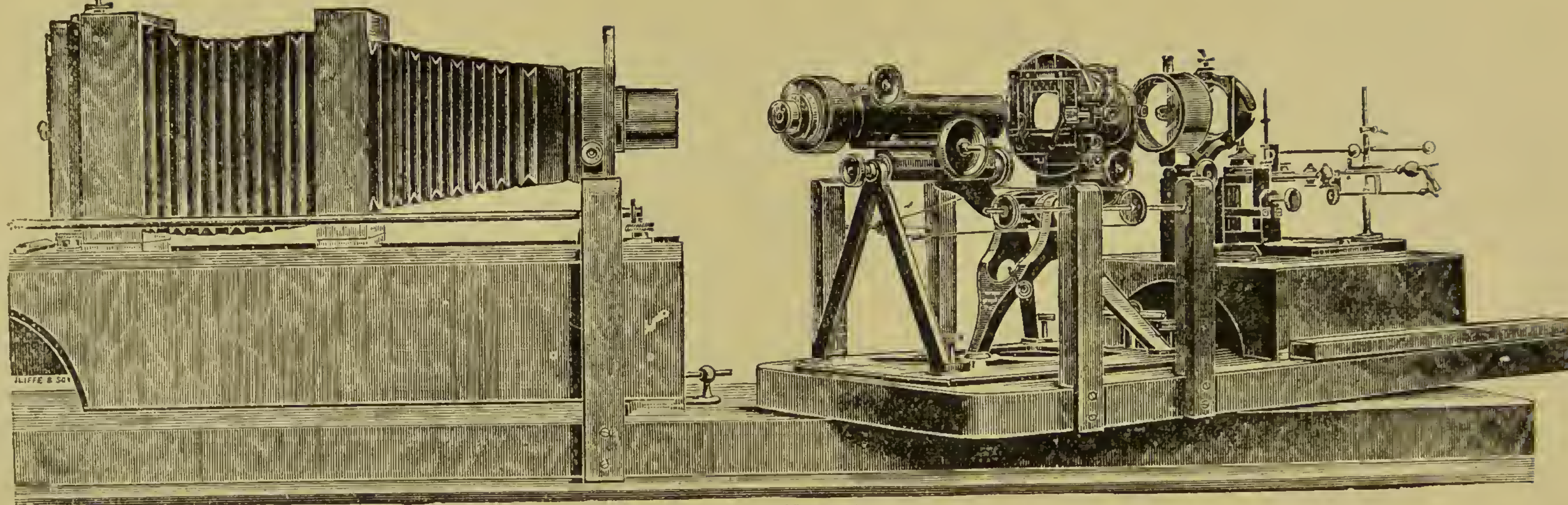


FIG. 8.

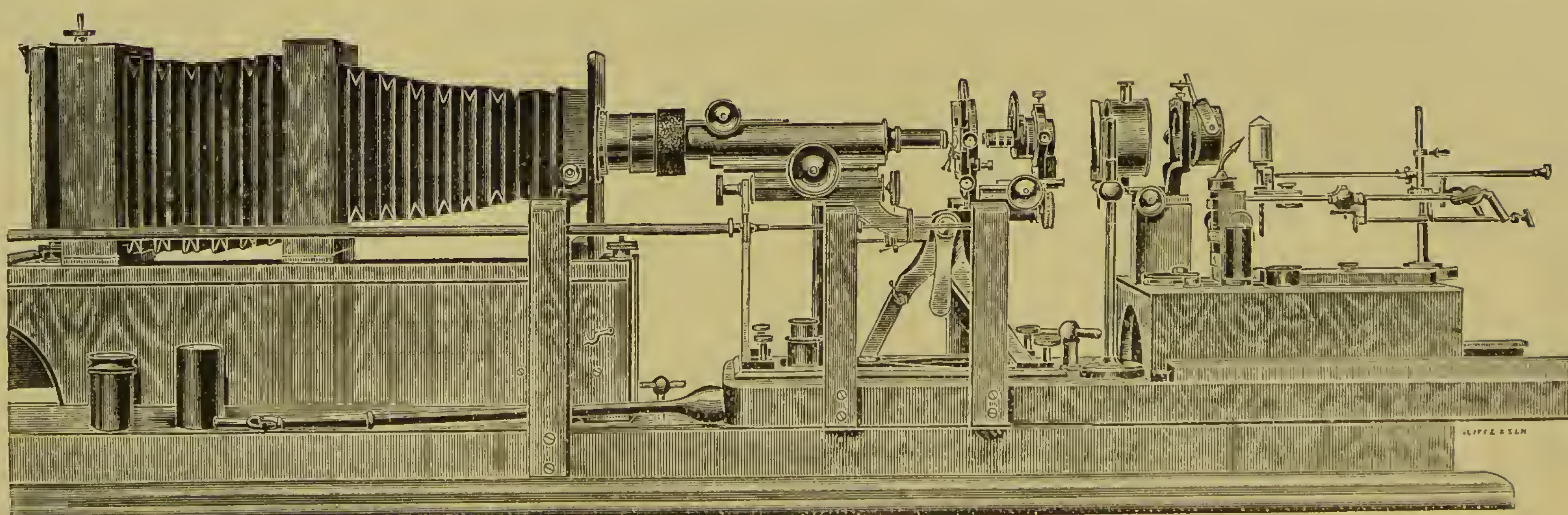


FIG. 9.

the ground glass simply to view the object in a general way, to ascertain if it is in the centre of our plate or not; we then put in its place a piece of plate, or other good glass, and focus the image carefully on this plain glass with an eye-piece of the Ramsden or Aplanat (Zeiss) type. The front of the plain glass should have lines marked on it with a diamond, and it will be found very useful to have these lines made at intervals of one-tenth of an inch, or such equivalent in centimètres as may be suitable. By means of these lines, the size of even very minute objects may be measured, almost at a glance, if the magnification at which we are working is known. The Ramsden, or aplanat, must, of course, be focussed once for all on the lines on the glass; if it is found difficult to do this, some such object as the wing of a fly may be fixed *pro tempore* to the front. Our friend, Dr. E. C. Bousfield, has recommended to us, and we use with great advantage, for focussing at higher magnifications, a double convex spectacle-lens of about 8in. focal length. This we have mounted in a rigid tube of such length that the lines are at its focal point, and we lay this on the plain glass and put our eye at such a point that the field of the lens is seen full of light; by this procedure we obtain a most accurate focus for high power work; but for low magnification the light is too strong for our eye, lime-light being used. While this is a description of our own instrument, it is to be understood that, if the reader keeps in mind the points we have laid down as absolutely essential to a good apparatus, he will, doubtless, find on the market other instruments worthy of his

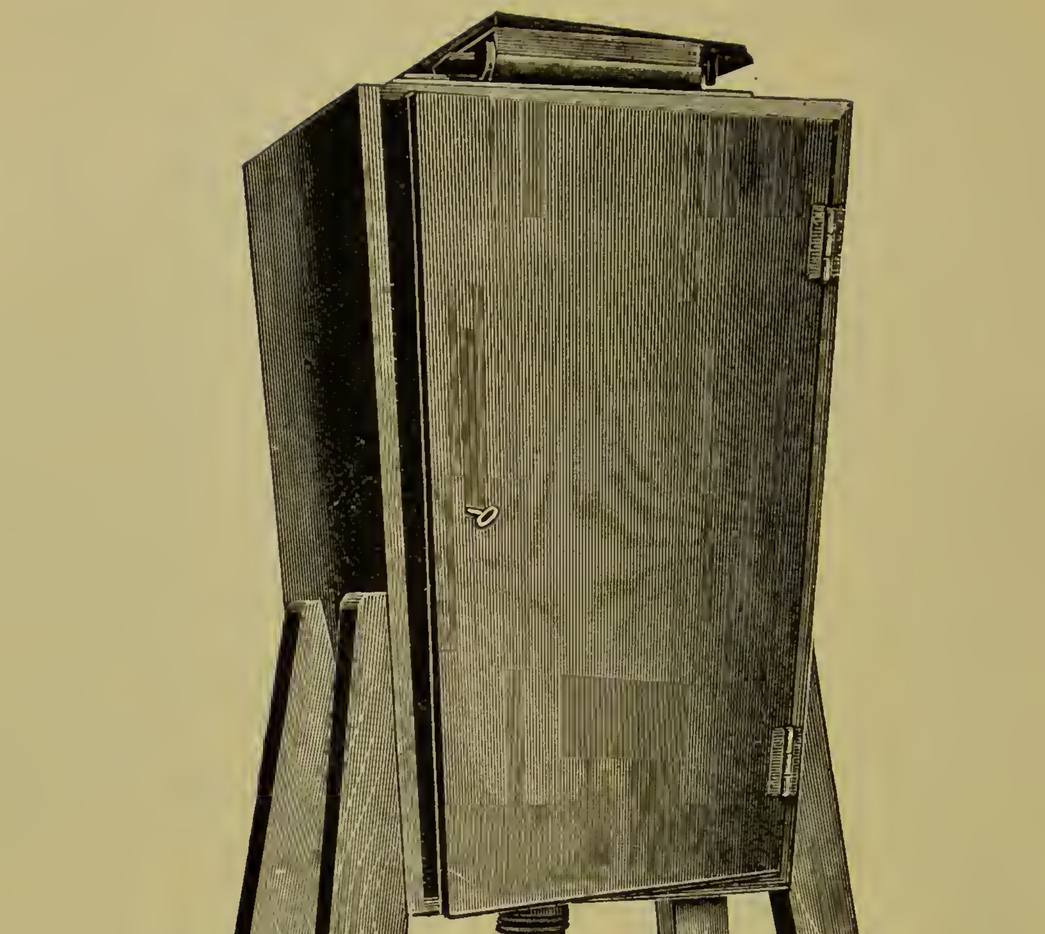


FIG. 10

confidence. Thus Messrs. Swift make apparatus less costly than that figured, and Messrs. Beck, Crouch, and Watson, in London, several in the provinces, and several on the Continent, may be trusted to supply apparatus well fitted for the work. But we venture to assert that any signal departure from the principles here laid down will be suggestive of imperfection in design.

VERTICAL APPARATUS.

Some first-rate authorities, among whom we may mention Dr. H. Van Heurck, of Antwerp, prefer to use, for general work, vertical cameras; while we fail to see the advantage of this for ordinary work, we are aware that it is sometimes necessary, as when we have to deal with liquid preparations, or such as cannot be covered or enclosed in a cell. And when we came to work out an apparatus to be used on the vertical we discovered certain great advantages hanging on this method which cause us to devote particular attention to the matter here.

In the first place, our apparatus, fig. 8, being clamped in all its parts to the base-board, can be quite well used vertically, and it has been so used with success. But we figure the apparatus of Dr. Van Heurck, which is made by Messrs. Watson for vertical use alone. It consists of an oblong box steadily mounted on four legs of such length that the ocular end of the microscope passes through the bottom of the box; and the box is large enough to admit the worker's head for focussing purposes. The whole front of the box opens, and the whole length of the "camera" is nearly 20 inches. The final focussing is done on plain glass at the

top of the box, where the plate is put for exposure (fig. 10).

Our own vertical instrument is a more pretentious affair than the above; but it is adapted for some unusual

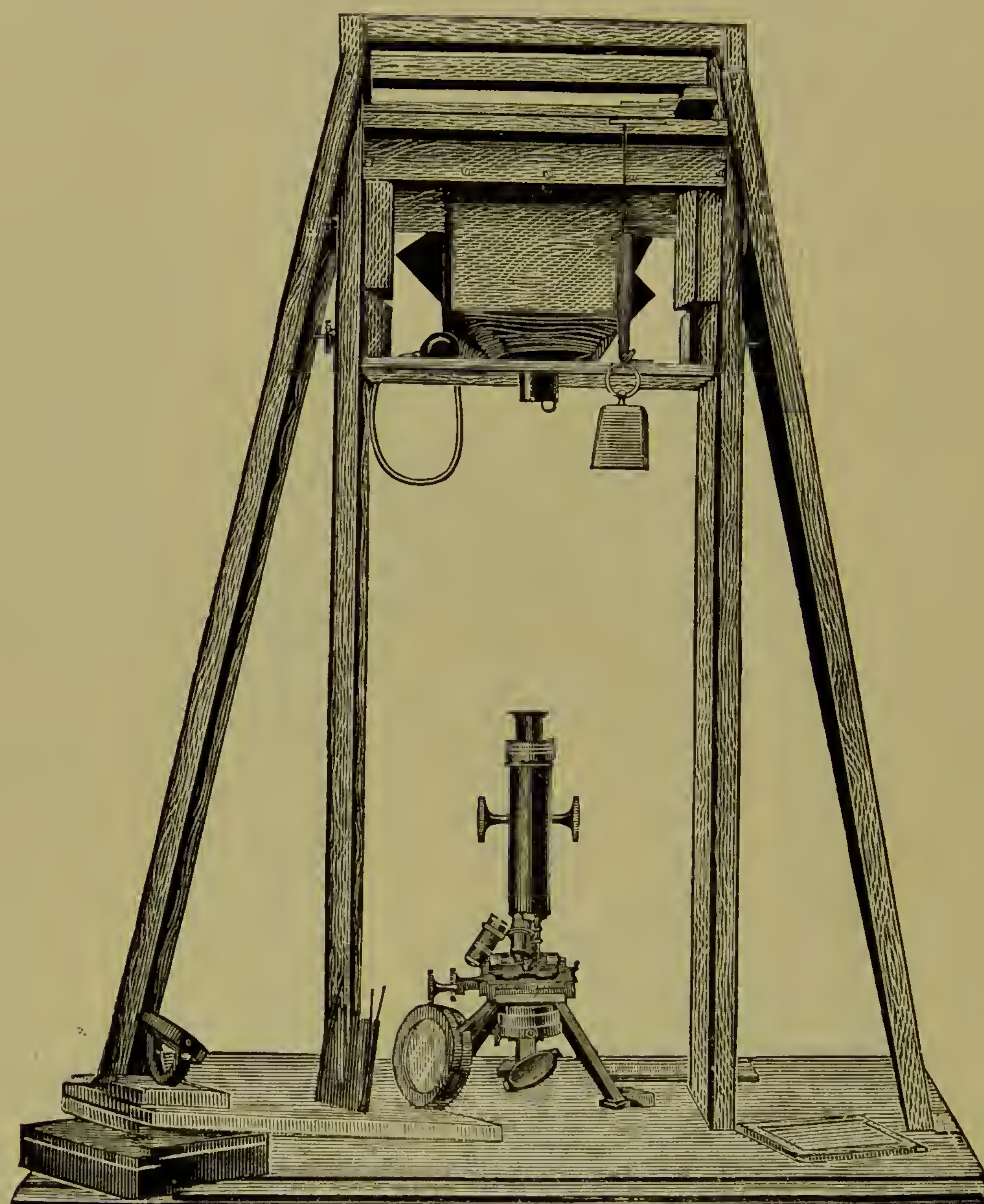


FIG. 11.

work. The whole is constructed with a view to absolute steadiness, for the instrument is used for the highest

power work. The base is 30 x 24 inches area and two inches thick, the side-pieces are also strong; except the struts, which are of brass, the whole framework is of wood.

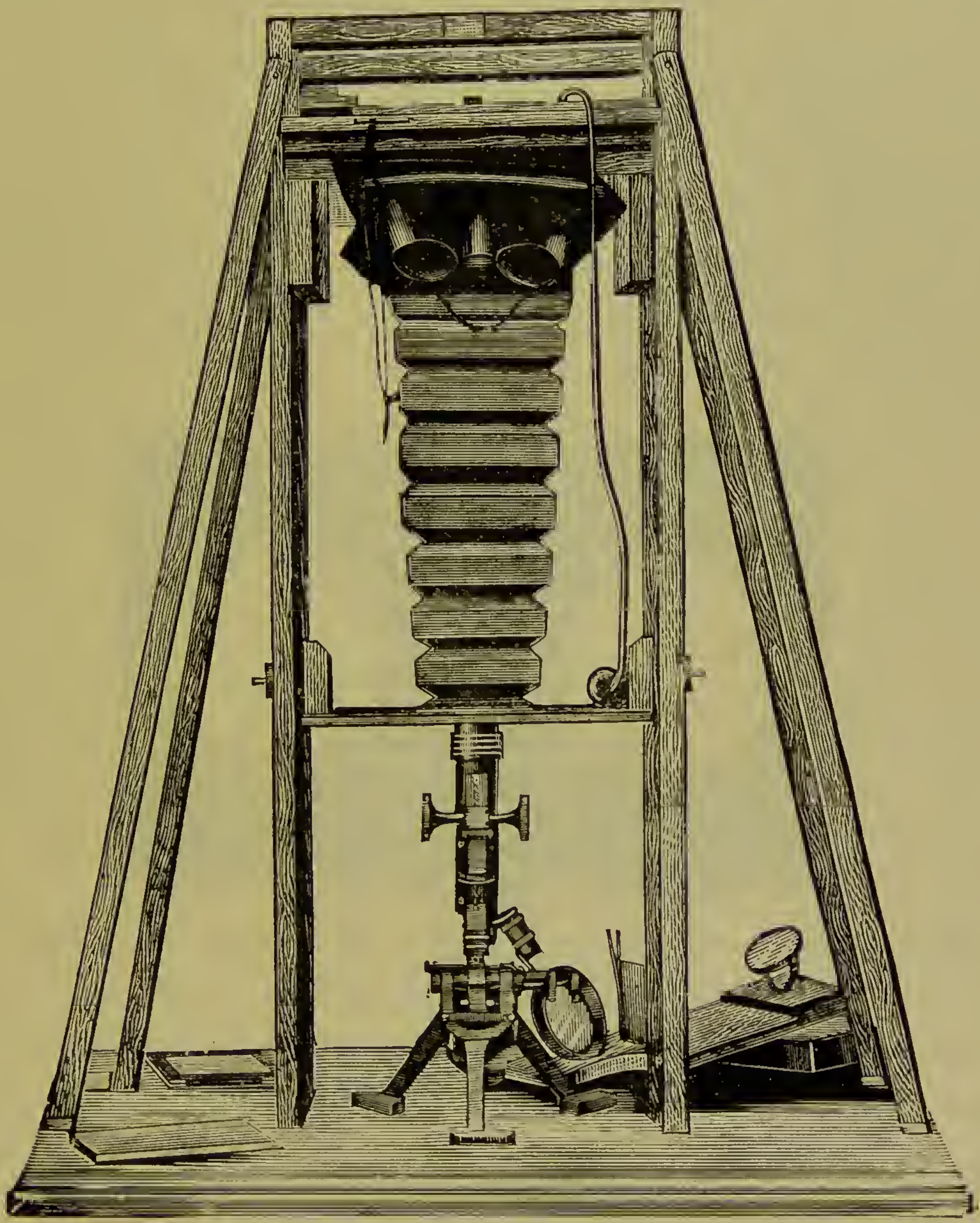


FIG. 11A.

Both back and front of the camera slide up and down the sides in slots; and the front can be pushed so far up that the head can be put below it for examination of

the image in the microscope in the usual way. The distance between the sides is 12 inches. The back carrying the dark slide is large, 15 x 12, not only for strength, but to allow of the use of an instantaneous shutter of the "guillotine" type. This moving shutter will be understood from the fig. 11B. It is made of sheet aluminium for lightness, and at AB is a slot half an inch wide which travels across the sensitive plate at a small distance, about one-sixteenth of an inch, or less. The plate size is $4\frac{1}{4}$ x $3\frac{1}{4}$ inches; the full stretch from ocular to plate, 26 inches.

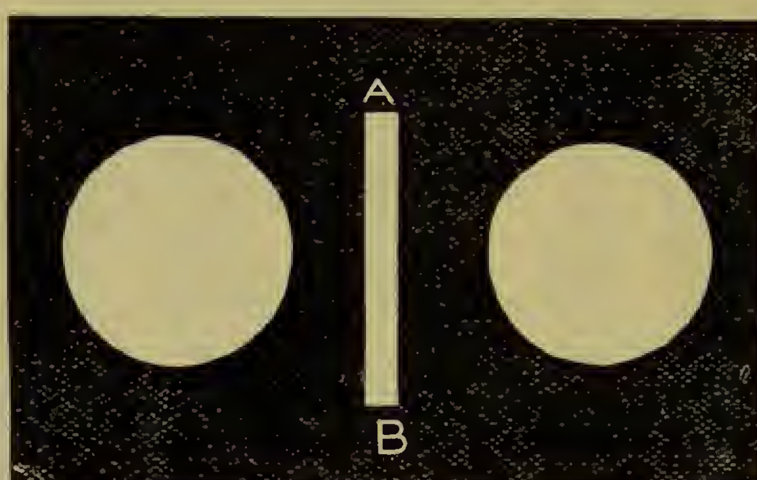


FIG. 11B.

The white discs are for the purpose of focussing by reflected light; the image on the disc is observed from an opening below and in front; to photograph moving objects we watch the white disc, the head being in a bag of velvet, or a bag of opaque material being tacked to the opening, and terminating in the frame of a pair of "goggles," seen in fig. 11A, held tightly to the face by a strong elastic round the head. Thus one hand is free to operate the focussing adjustment of the micro-

scope, the other to release the shutter at the proper moment. Various ways of actuating the shutter have been tried, the best seems to be by a weight calculated to suit the desired exposure.

As made, this shutter in action does not shake the instrument, but by causing the shutter to work on wheels or balls the friction of the movement may be lessened, and a less ponderous instrument may probably be used. For low power work, where no ocular is used, it is essential that there shall be the minimum of space between the shutter carrying the focus disc and the plate, and the shutter must be thin; but even if these precautions were not sufficient the camera-back might be lowered to the necessary extent at the critical moment by a very simple device. The weight actuating the shutter is attached to the shutter by a length partly of cord, partly of elastic; this gives a very "sweet" motion, without jar or violent stop which might damage the fittings. The system of making the exposure close to the focal plane is the best for securing the greatest efficiency of exposure; the greatest amount of light action available during exposure is brought to bear on the plate. By the use of another back the focus can be arranged for still objects in the usual way from the back, or top, of the apparatus.

As we are able to watch the lighting on the focus-plane up to the last moment, this apparatus obviates the necessity for a heliostat in cases where short exposure is required; and with direct sunlight the exposure need not ever be long. The illuminating apparatus seen in our fig. 11 is so placed that the mirror can be reached by the hand while the operator is watching the image on the white disc;

consequently, the light can be centred and the exposure made before the light has had time to be decentred to any appreciable degree. This illuminating apparatus consists simply of a mirror, a diaphragm, a paralleliser, and, when desired, a glass or cell for monochromatising more or less the light. As shown in the figure, it is only in an experimental stage of manufacture. For some of the ideas in this apparatus we are indebted to our friend, Mr. H. S. Starnes, and in the construction Mr. C. L. Curties has aided greatly by his ingenuity. This experimental apparatus was made in the workshop of Mr. C. Baker.

We find, on further experiments with the shutter above described, that it answers its purpose admirably, except that, as the velocity of the falling weight increases as the weight falls, we get a corresponding increase of rapidity in the exposure as it proceeds. Accordingly, it will be found better, and we propose at once, to actuate the moving shutter by a spring, such as an elastic band stretched. Of course, this may lead to a jar at the moment of release, but we find our apparatus sufficiently strong to withstand a moderate amount of such action.

We also have experimented with a right-angled prism placed behind the objective in such a way that the image can be observed through a tube at right angles to the ordinary microscope tube, the sensitive plate being meanwhile uncovered but protected from the image rays by the prism. The prism could be rapidly moved to one side by a spring, and so an exposure could be made at a given instant. This principle may be found in a photo-micrographic apparatus of Nachet, and is described by Van Heurck, but in certain details we were unable to make it wholly satisfactory.

CHAPTER IV.

Objectives and Oculars.

AS a matter of course, the greatest care should be taken to provide oneself with the best optical appliances that can be procured; but the difficulty in choice which existed a few years ago is now practically overcome by the advance in optics that has been made. It is now more a question of expense than anything else. In past times, objectives were made excellent in every respect for ordinary ocular observation, but not "corrected for photography." It is well known that in consequence of the unequal refrangibility of those parts of the spectrum which chiefly exercise chemical activity, and of those parts which chiefly act upon the retina, the foci of the chemical and visual rays passed through a lens do not correspond in position; that, while an image might appear accurately in focus to the eye, it might not impress itself in accurate focus on a photographic plate. During years now passed, objectives were made and called "achromatic," but seldom was attention given to eliminate this defect, which did not interfere with the performance of the objectives for visual observation; but when attention began to be paid to photo-micrography, opticians were forced to make objectives in which the actinic and visual foci should be coincident. At first, this was only imperfectly achieved, for, with

the samples of glass then obtainable, it was impossible to correct objectives so as to make all the spectrum rays coincide in focal point; and objectives would be corrected for only two sets of rays. Let us suppose that a given objective was corrected so that the blue and yellow rays should come to focus in the same plane vertical to the optical axis; then the red and green rays would still focus on some other plane, and this fact would be demonstrated by the existence of coloured fringes round the image, the fringes in this particular case being red and green to the eye, and represented on the photographic plate by more or less blur. In spite of this defect many of the objectives made just before the introduction of "apochromatic" objectives gave good results in photo-micrography, but never such results as are obtained now with apochromatic glasses, or even with achromatic glasses or orthochromatic plates. Experiments conducted in Germany by Dr. Abbe and his colleagues, and aided in the most laudable manner by the German Government, resulted in the production of many samples of glass "metal," some of which have such qualities of *density* and *dispersion* that Abbe was able to calculate objectives to be made of some of these glasses, wherein corrections can be made so as to practically bring the entire visible spectrum to one focal point. Techically, Abbe was able by objective and ocular combined to eliminate the whole of the "residual spectrum," which residue was previously indicated by the fringes and the blurring mentioned. And we have, further, objectives midway in quality and price between the old achromatics and the new apochromatics, the latter being of necessity

very expensive in comparison with ordinary achromatic lenses.

In the matter of "correction," then, we may take the achromatic as the lowest class of objective; the semi-apochromatic as next; the apochromatic as the finest objective at present obtainable.

Certain facts with regard to the use of ordinary "uncorrected" achromatic objectives for photography have lately been brought to light, and these are of such vital importance to the photo-micrographer who is restricted as to pecuniary outlay, and who requires only good definition irrespective of abnormally large aperture, that serious attention must be called to these facts.

The matter seems first to have been mooted by Dr. Piffard, of New York, who noticed that with "uncorrected" objectives, microscopical and photographic, he obtained sharp images, provided he used orthochromatic plates and certain "screens," or light filters. Mr. T. F. Smith claimed to have gone a step further, for he stated that the colour-correct plate was enough without the screen; but as he used an oil lamp, he was really using a "screen" more or less yellow. The writer made numerous experiments with a view to test and bring together the experiments of these gentlemen, with the result that he made the following statement. Many, if not all, ordinary achromatic objectives, which on ordinary plates give a blurred image in photographic work, will give a sharp image when an orthochromatic plate is used. This improvement is increased by the use of such a light-filter or screen as will cut off more or less of the violet and blue regions of the

spectrum ; the yellow glasses supplied by the makers of "Isochromatic" and other colour-correct plates are found to answer well. Further, certain objectives intended to be used with a projection or other ocular, which do not give a sharp image on an ordinary plate when the ocular is not used, will give a sharp image on an orthochromatic plate under the same conditions. Mr. E. M. Nelson finds, moreover, that even the apochromatics work distinctly better on colour-correct plates than on ordinary. And Mr. A. A. Carnell has demonstrated to the writer that he gets better results with balsam-mounted objects, or objects in any of the yellow coloured media, than with objects mounted dry.

Briefly, objectives, so-called achromatic, of old and cheap types, will frequently, if not always, give sharp photographic negatives on orthochromatic plates, though they may not do so on ordinary plates ; a screen cutting off violet and blue rays "helps out" the correcting influence of the plate ; and we are to take as tantamount to a yellow screen any substance in the light-way, or any quality in the illumination, that makes the light acting on the plate more or less yellow, or green-yellow. *But* these facts do not in any degree help us in regard to wide aperture ; the spherical aberration of an objective is not counteracted in any way by colour-corrected plates ; and for the most critical and perfect work we still require objectives of the apochromatic type.

Objectives have been produced called "semi-apochromatic" by some Continental opticians, notably by Leitz and Reichert, and we can corroborate the high opinion of these lenses given in Dr. Dallinger's edition of "Carpenter."

The editor of that book speaks highly of several objectives of this type, and he says of one—Reichert No. 6—that it is the "rival of even true apochromatics."

Briefly, we recommend those who aim high, and who can afford them, to use apochromatic objectives; failing these, semi-apochromatics; but good work can be done, sufficiently good for many purposes, if not for most, with well-chosen achromatics of the most modern construction. The vast majority of apochromatics are made by Zeiss, but we are informed, by those on whose authority we rely absolutely, that Powell and Lealand make apochromatics not inferior, and often superior, to those of Zeiss at a somewhat lower price.

Definition—and consequently "resolution"—depends on "numerical aperture." Definition is the highest quality an objective can possess, and so we ought to look for high aperture in our lenses. But the *crux* of practical optics is to get high aperture with low power. "Power" depends on the focal length of the lens; the shorter this length the higher the magnifying power. It is easy to get high angle with high power, but the converse is the most difficult problem the optician has to face. Lenses are frequently quoted as having a high angle of aperture and a low magnifying power; when put to the test they will be found to be of higher power than stated.

"Penetration" is an imaginary quality supposed to represent the power of giving at the same time a sharp focus on two separate planes at right angles to the optical axis, which is plainly absurd. A lens may be of such nature that no plane is thoroughly sharply focussed, and

so the eye may be deceived into an impression of sharpness on several planes, simply because it finds no sharpness to compare with blur. Yet this want of sharpness on any plane, and impression of sharpness on several planes, may have its value, however small, in certain branches of research, where only general appearances are the object of study. But in photo-micrography all blur is fatal to success. Compromises in sharpness between one plane and another will not do, we must have the greatest possible sharpness on the critical plane at least; and, further, this is the best plan for getting crisp definition as nearly as possible over all, as will be found by anyone who makes careful comparison between wide and narrow angled lenses at the same magnification. The blurring, or "confusion," due to depth of subject, increases as the aperture increases, but it increases as the square of the magnification. As it is of the utmost importance that this should be thoroughly understood, we put it in the words of Dallinger's "Carpenter." "If a transverse section of an object is magnified 100 times in breadth, the distance between the planes of parts lying one behind the other is magnified 10,000 times at the corresponding parts on the axis when the object is in air, 7,500 times when it is in water, and 6,600 times when it is in Canada balsam." It is, therefore, evident that the best results will be obtained by using as low a power and as high an angle as possible. This combination of wide aperture and low power, together with good corrections of spherical and other aberrations, is found in the apochromatic lenses in the greatest degree, the semi-apochromatics coming next in this respect.

Practice as well as theory proves this position ; for it will be found that much better results will be got in observation, as in photo-micrography, by using a magnification as low as consistent with the eye's power of vision, and an aperture as high as consistent with perfection in optical mechanism. This remark applies as much to physiological and histological research as to any other branch, though physiologists seem to be the slowest to recognise the truth. To separate or "resolve" details of a given fineness, or at a given distance apart, requires a given aperture ; if a lens has not sufficient aperture to effect the resolution, no amount of magnification will cause that lens to show us the details clearly separated. And every advantage lies with definition as against mere magnification ; for with a low magnification, provided it is enough to make the details large enough for our vision, we tax our eyes less, we have less of the blur caused by the planes lying behind and in front of each other, and we see more of the surroundings of the particular detail under observation, or we have a larger "field" of view at one time. The photo-micrographer, then, and, indeed, every microscopist, is urged to use low power glasses when possible, and as high numerical aperture as is consistent with general good quality in the objective. It is not proposed here to discuss the exact meaning of the term "numerical aperture"; it must suffice to say that it not only represents a mathematical fact, but is an efficient method of comparing the effective apertures of dry and immersion lenses. The highest possible "N.A." in air is 1 ; in water, 1.33 ; in cedar oil, about 1.52.

If an object is mounted in the usual way between two *laminæ* of glass, and if that object be examined with a "dry" objective, air being between the cover-glass and the objective, some light-rays are reflected back from the under surface of the cover-glass, and do not emerge from the preparation at all. Other pencils coming out of the denser medium (glass) into the rarer medium (air) are refracted away from the perpendicular to such an extent that they never reach the objective, and so are lost. But if for air we substitute the denser medium (water), or the still denser medium (oil), the rays are less and less refracted on emergence, and, in fact, reach the lens instead of passing into space. In the words of Mr. E. M. Nelson, our valued instructor and friend, the "fan of diffraction is closed up by immersion." A very simple diagram will illustrate these points.

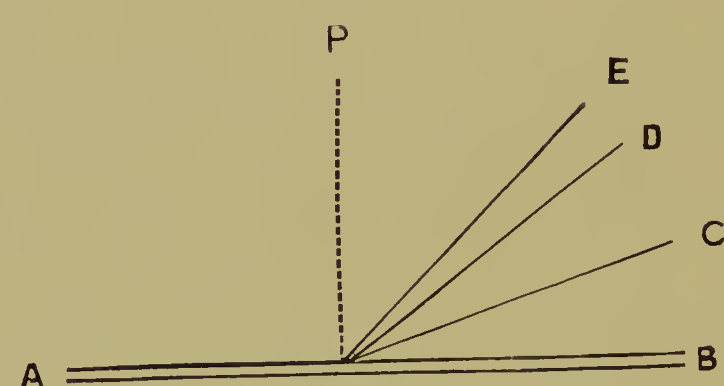


FIG. 12.

A pencil emerging from the cover-glass A B into air may pass towards C, and the objective may not grasp it; in water the direction may be towards D, in oil towards E, and evidently an objective may lose C and grasp D, or may lose C and D and grasp E.

Water immersion objectives are not nearly so much used as oil immersions, or—as they are not quite accurately called sometimes—"homogeneous" immersion objectives. The practical advantages of the immersion system are numerous and grave. Much greater aperture can be obtained, more light passes through the lenses, greater "working distance" is possible. Working distance is simply the distance between the front of the objective and the top of the cover-glass when the object is in focus; but it is of some importance, for we have known even immersion objectives with so short working distance that we have been unable to examine preparations which happened to have cover-glasses slightly thicker than usual. Objectives up to the eighth of an inch are generally made "dry"; eighths are sometimes dry and sometimes immersion. We have a dry 3 mm. glass of Zeiss (apo. $\frac{1}{8}$), and also a lens of equal power, apo. oil immersion N.A. 1.4; each lens is excellent for its own purposes.

Objectives of higher power than half-inch are usually made with "correction collars"; these collars are for the purpose of altering the relative position of the combinations of the lens, to meet various thicknesses of cover-glasses. In practice the collar is of great importance towards securing the best definition, and careful study and practice should be devoted to the use of the collar. But in the case of the apochromatic immersion glasses of Zeiss, the correction for cover-thickness is achieved by alteration of the length of the optical tube, as has already been mentioned. In selecting objectives it is necessary to be careful as to the power quoted by the maker, not only for

the reason given above—p. 53,—but on account of the fact that one objective will stand the use of a more powerful ocular than another objective may stand. Thus, when we compare a one-sixth with a quarter-inch, we may find that the quarter will give a good image with a high power ocular, while the sixth will break down under the same ocular. In fact, there is no better rapid test of an objective than to use it with a strong ocular. Oculars in this country are marked by arbitrary letters, A, B, C, etc., A being the lowest power; the system of Abbe or Zeiss is far preferable, for their oculars are marked with numbers showing their actual magnifying power. Thus, a Zeiss "compensating" ocular marked "8" magnifies the objective image eight times, and this conveys a definite meaning to us.

There is some difference of opinion as to the use of the ordinary Huyghenian eyepiece for photo-micrography; but there is no doubt, first, as to the advantage of an ocular for this purpose when the result is a good image; and second, as to the excellence of the projection oculars of Zeiss. The use of a suitable ocular lessens the length necessary for the camera, and prevents much of the danger of stray light reaching the plate. Good photo-micrographs have been produced with an ordinary ocular, but we have not succeeded with it, and we cannot but suspect that the successes of others have been fortuitous. Certainly, the ordinary eyepiece is not intended for this purpose; but, on the other hand, many fine results have been achieved with Zeiss' compensating oculars, which were not intended for this work any more than the Huyghens' oculars. At any rate, the projection oculars are strongly recommended for photo-

micrography, and the compensating oculars may be found handy if, at any time, the projection does not happen to give the desired magnification.

It was found impossible to remove certain defects from the high power apochromatics without help of oculars, while these defects could be easily removed from the low powers; consequently, to obviate the need for a great many oculars, Zeiss leaves in his lower powers these defects, which are corrected by his compensating oculars; and it will be found that the apochromatics do not perform well for photo-micrography without either a projection or a compensating ocular. But most of the achromatic lenses of modern make answer very well without eyepiece; we are in the frequent habit of using several glasses in this way. Two fine very low power glasses by Zeiss are made for use without ocular—the 70 and 35 mm.—and Zeiss' *aa* is also most valuable for use alone. In this way we have used also low power glasses by Swift, Beck, and Crouch. Zeiss makes projection oculars numbered "2" and "4" for the short tube stands, and "3" and "6" for the long tube. These oculars work well with many lenses not apochromatic that have been in our hands.

We may call the magnification given by an objective at the distance of ten inches from its posterior conjugate focus the "initial power" of that objective; and for guidance it may be stated that a *good* objective will stand the test of photography at about six times the initial power; a *very good* glass will stand ten times; nothing but a *very fine* glass indeed will tolerate being strained more than this, and it does not matter how the

tax of magnification is brought about, whether by stretch of camera, by heavy eye-piecing, or by these combined. Thus, the initial power of a Zeiss apo. 3 mm. glass is 83; as soon as we try to make a negative at about 800 diameters, we begin to find difficulties, which only a master of the science can overcome. It will be found that, for the above reason, the No. 6 projection ocular is difficult to use except with a short camera, but, so far as we can discover, a given magnification can be obtained as successfully with the No. 6 ocular and a 12 inch camera as with the No. 3 and a 24 inch stretch. The camera of Dr. Van Heurck does not stretch beyond about 20 inches, yet no one is likely to find fault with his results on this score.

A moderate battery of glasses might be as follows:—A 70 mm. Zeiss; a one inch, by any good maker, achromatic; a quarter-inch, such as the apo. 6 mm.; and an immersion twelfth. The following might be considered a complete armament:—70, 35, 24, 12, 6, 3, and 2 millimètres, or their equivalents in English measure. The two finest and most useful glasses the writer has ever possessed are the 12 and the 3 mm. apochromatics, and next to these the *aa* achromatic for use without ocular. Of oculars, the No. 8 of Zeiss is very useful, but ordinary achromatic lenses begin to break down even visually under this ocular, so that for these lenses it is well to have an ocular of lower power. The projection ocular may be chosen according to the length of tube and of camera intended to be used. In view of the facts stated with regard to the use of orthochromatic plates with "uncorrected" lenses, it may be pointed out that very fine results, where the greatest

apertures are not required, may be obtained with good achromatic glasses, provided colour-correct plates are used with the addition of the "screen" when necessary. This greatly lessens the pecuniary outlay necessary to produce the finest results usually required for medical, biological, or mineralogical work.

CHAPTER V.

The Condenser: Its Fittings and its Use.

NEXT in importance to the objective among our optical appliances comes the condenser. The paralleliser or bull's-eye is sometimes called a condenser, and is sometimes used really as a condenser, but throughout this book, when the term "condenser" is used, it is to be taken to mean the "substage condenser."

It is to be noted that, without a good condenser, the good qualities of the best objectives are lost; the function of the condenser being to furnish a cone of rays of such angular compass as could not be obtained without some condensing arrangement, and such as is appropriate to the angular aperture of the objective used. It will be found that in all cases the angle of the cone projected by the condenser ought to bear some fairly close relation to the aperture of the objective in use. If we use a cone of large angle with an objective of low angle we shall destroy the performance of the latter; and if we use an objective at a large aperture with a condenser of low aperture, we simply throw away the most valuable quality of the objective.

The concave mirror serves as a very low-angled condenser when the object is in its principal focus. To give facility for this disposition, the mirror of the microscope must slide up and down the rod or "tail" to which it is

attached; and it is pointed out in Dallinger's "Carpenter" that with diffused daylight even the plane mirror can be used to "condense."

A bull's-eye may also be used as a condenser up to an angle of about 25 degrees; to effect this we turn the convex side of the lens towards the light, and bring the lens near to the object; in such a case, looking at the back of the object and moving the bull's-eye to and from it, we can see when the latter is in the proper focus.

Sometimes in the very lowest power work even the mirror or the bull's-eye gives too small a field of light; in such a case we interpose at a short distance behind the object a piece of finely-ground glass, using parallel or slightly diverging rays from a paralleliser or bull's-eye. This will be found a good help out of a difficulty which not seldom turns up in general work.

The next form of condenser requiring attention is the ordinary achromatic made by all opticians. One of these may be used giving an angle of about 160 degrees, and it should be so constructed that the top element can be removed, the remainder giving angles suitable for objectives such as one inch, two-thirds, or ordinary half-inch.

The most useful of all types of dry condenser is represented by the achromatic of Zeiss, having numerical aperture available to about ·95. The front of this may be removed, and the remaining part forms an excellent condenser for the lower power objectives. This is the condenser most frequently used by the writer and by several of his friends.

This condenser is shown in fig. 13.

Higher in the scale of excellence and of expense is the apochromatic condenser of Powell and Lealand, avail-

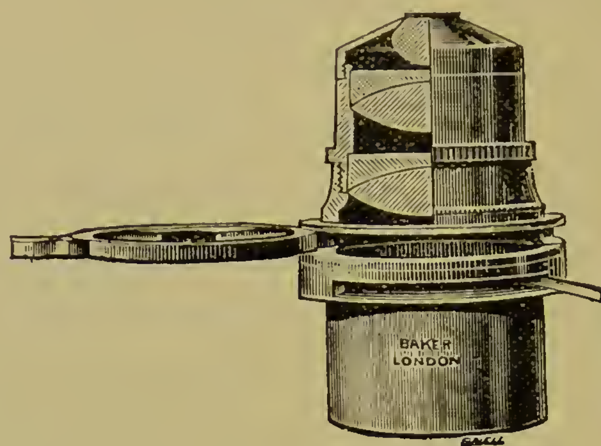


FIG. 13.

able N.A. nearly 1. For ordinary medical and similar work this condenser is unnecessarily costly, but for critical work it is desirable (fig. 14).

FIG. 14.

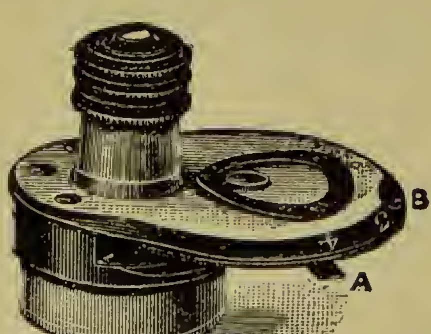


FIG. 14.

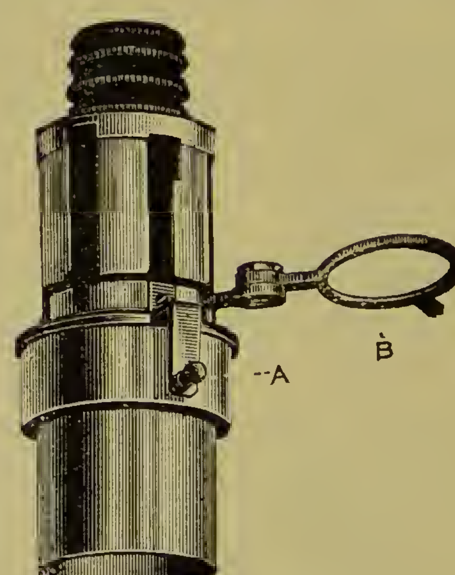


FIG. 14A.

Those who propose to use immersion condensers are presumably ambitious of the very finest results, and for this we recommend the best condenser at present known to us—the achromatic oil-immersion of Powell and Lealand, N.A. 1·4. This is not usually fitted with an iris stop, but it is altogether as near perfection as anything that can be obtained. It is costly—see Appendix—but is not dear (fig. 14A).

A very important point not usually noticed in books is that good objectives may be used as condensers with the best results. All that is needed is a fitting of such nature that the objective can be screwed into the substage, and an iris can easily be added to the arrangement. In this way we have produced excellent results, using as condensers both achromatic and apochromatic objectives; the chief point to note is that the condenser-objective should be about the same N.A.—less rather than more—as the objective used. Dr. Bousfield has used two immersion objectives, one as condenser, the other as objective.

Chromatic condensers are less expensive than achromatic or apochromatic, and the cheaper ones act fairly well in general work; at the same time, considering that good achromatic condensers are not so very costly, we do not advise the purchase of chromatic. Zeiss makes two chromatic condensers, one for air, the other for oil apertures—both are fairly good, and neither is very expensive—but we would prefer, for general work, the dry achromatic mentioned above.

Various "stops" are supplied with a condenser; some of these may be seen in fig. 14. The stops opaque in the

centre are for providing either a "hollow" cone of light, or for "dark background" work; the others are for oblique lighting, of which more shall be written later in this book. Moreover, there are in existence, though practically out of use, various kinds of illuminating apparatus which may come under this head of Condensers; for instance, there are "spot lenses," "parabolic reflectors" for use above the stage, and others. The appliance known as a "Lieberkühn" is interesting, and in a few cases useful, and shall be briefly noticed later. Regarding the

Use of the Condenser.

Fig. 15, from Mr. Nelson, shows the illumination when a condenser and an objective of equal aperture are used together, the condenser being in focus; here the object lies in the plane of the conjugate foci of objective and condenser; and if we look at the back of the objective we shall find it wholly illuminated. But if we cut down the aperture of the condenser by any means we shall get the state shown in fig. 16, where part of the aperture of condenser and objective is lost.

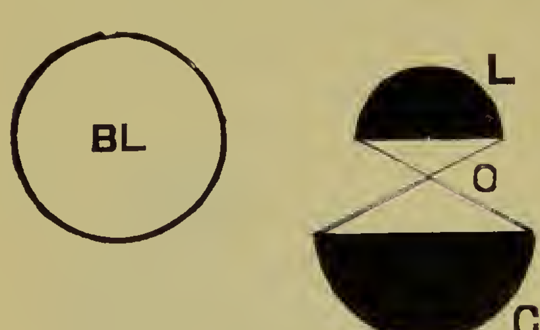


Fig. 15.

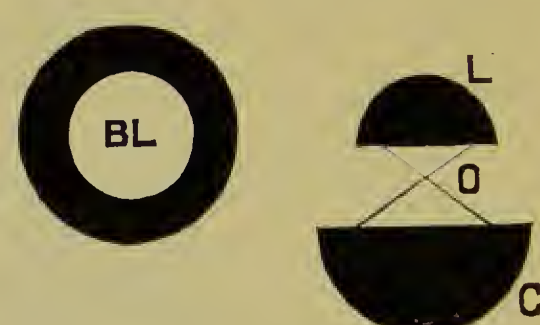


Fig. 16.

In these figures C is the condenser, L the objective, O the object, and BL the back combination of the

objective, as seen down the tube without the ocular. Sometimes it is found that when both objective and condenser are in the proper focus, the area of even illumination is not large enough to cover the field, and to overcome this some workers deliberately put the condenser out of focus, generally by racking it down. This certainly spreads the light out over the field, but for two reasons the practice must be condemned. First, a certain amount of the aperture of the condenser is lost, which may or may not matter; but second, the state of affairs shown in fig. 17 is brought about, and this is clearly wrong, for the object no longer lies in the focus of the condenser.

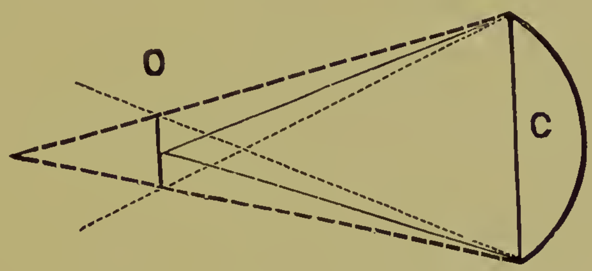


FIG. 17.

O is the object, C the condenser. Continuous lines represent the condenser in focus; dotted lines the condenser racked downwards; interrupted lines the condenser racked upwards.

If the condenser is racked down, as shown by the dotted lines, the rays cross before they reach the object; if it is racked up, they cross above the object; and in either case the field is more evenly lighted, but damage is done. If, when the condenser is in focus, it is found that the field is not evenly lighted all over, the

proper plan is to use a longer focus condenser, or to bring the radiant nearer if no paralleliser is in use, or to use a paralleliser.

The iris diaphragm, be it noted, though much more convenient to use in a condenser, is not so accurate a device as the ordinary plate with apertures properly cut. The iris aperture is not strictly circular, the "leaves" do not answer perfectly to the actuation, especially at the small apertures, and it is difficult to set the diaphragm accurately to any desired aperture.

CHAPTER VI.

ILLUMINATION.

SUNLIGHT is undoubtedly the best radiant for the photo-micrographer, when he can get it. But in this country direct sunlight is so rarely to be depended upon, that if we were to provide apparatus for the utilisation of this light alone we might yearly pass many days without making a single exposure. Diffused daylight, however fine in quality for general photography, is of little service to the photo-micrographer, who requires rays more or less direct. But direct sunlight contains so large an amount of the rays most useful to us, and the rays most suitable for photographic purposes are so easily separated out from the other rays, a large amount of photographic light being still available for even very short exposures, that sunlight should not be wholly overlooked in a manual such as this.

If we propose to use sunlight we shall almost certainly require a heliostat of some kind. This instrument enables us to keep a reflected image of the sun shining steadily in one direction in spite of the apparent motion of the sun. The heliostat (fig. 18) is as good as any we have seen or used.

Next to sunlight in intensity and in actinicity comes the electric arc lamp. This light has the advantages named, but it is somewhat awkward to use unless a

"bull's-eye" or paralleliser be placed between the radiant and the condenser, as otherwise we shall get an unpleasant image of the arc on our negative when the condenser is properly focussed. Moreover, this light is apt to be very irregular in its intensity, the carbons never being really homogeneous throughout their length. As, however,

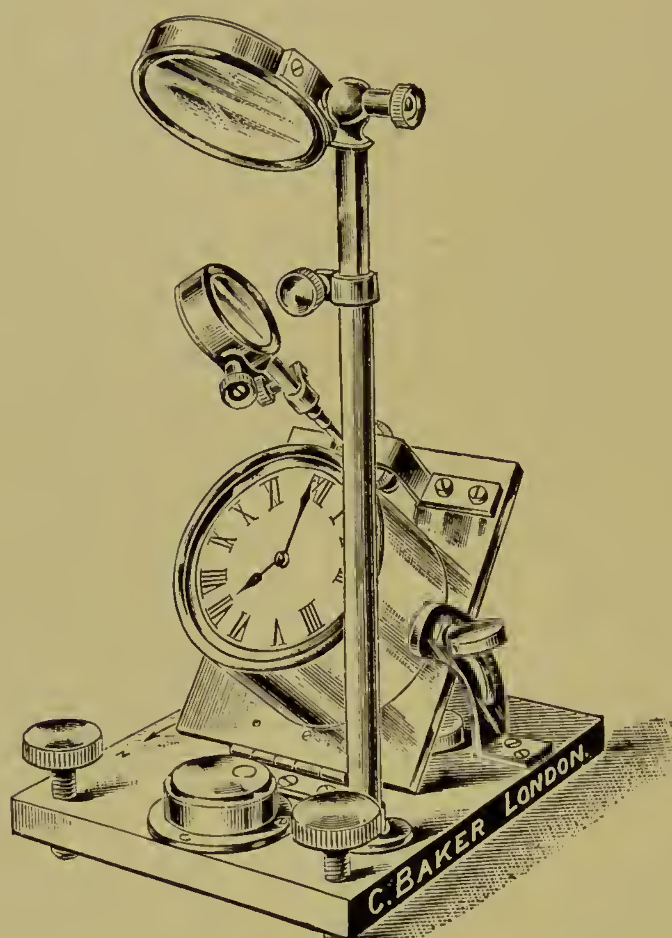


FIG. 18.

those who have the current may wish to use this light, we give a figure of the lamp which seems to answer the purpose better than any other form we have seen. It is used at the Conjoint Laboratory of the Royal Colleges of Physicians and Surgeons, to the Director of which, Dr. G. Sims Woodhead, we are indebted for the figure.

Another electric arc lamp worthy of notice is the Brockie-Pel, as sold by Messrs. Newton and Co. An incandescent electric lamp may also be used, as it is by Dr. Van Heurck, but here again, unless a paralleliser is introduced, we shall get an unsightly image of the filament under certain conditions.

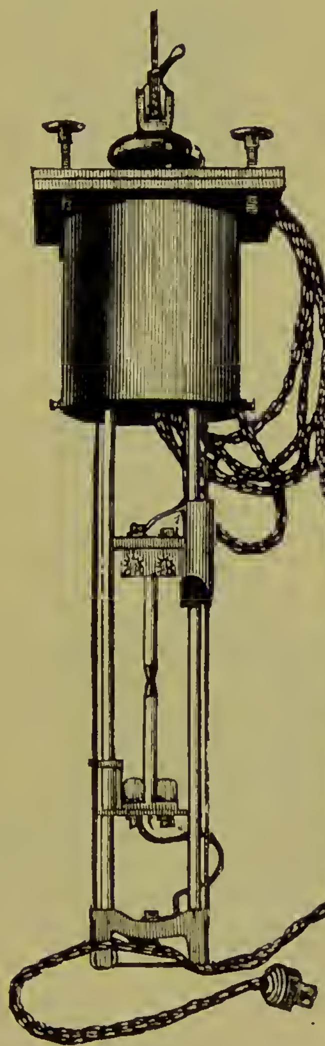


Fig. 18a.

On the whole we may fall back upon two radiants, both of which are well known, an oil lamp and the lime-light. The lime-light is much more intense and actinic than the oil lamp, and shortness of necessary exposure is a grave consideration, not so much for economy of time, as for

lessening the danger of tremor, and of the parts of the microscope being displaced during exposure by changes of temperature and other causes. Roughly speaking, a good oil lamp will entail about thirty times as much exposure as a good lime-light.

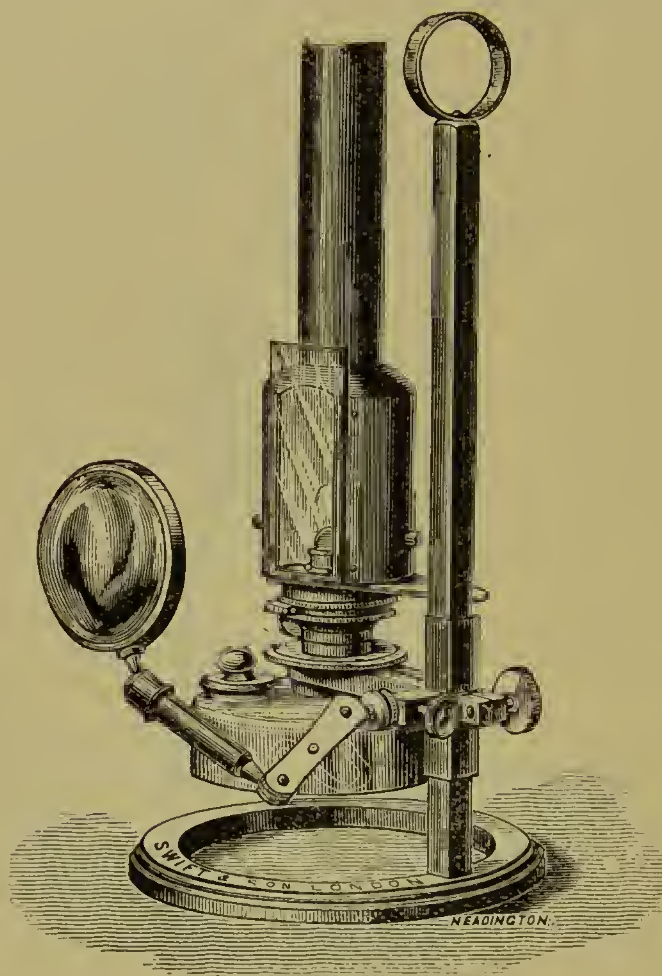


FIG. 19.

If oil light is to be used it is well to have it as powerful as possible; the ordinary lamps used in microscopical observation are by no means powerful enough. Our lamp should have a wick at least one inch wide, and the writer prefers a flat wick to a round one, though some excellent work has been done with the round wick.

The lamp recommended is made by Swift, and is shown in fig. 19.

About an ounce of camphor added to a pint of the best paraffin will produce a light more than usually white and actinic. The slip of glass in front of the flame is apt to break, especially when the flame is turned edge to the microscope as we recommend. To prevent this mishap the slip should be boiled for an hour in water or, better, in oil, the slip being wrapped in a cloth during the boiling.

The lime—or oxyhydrogen—light is more costly to inaugurate, but we find it the most satisfactory of all the illuminants. It can always be relied upon; it is very equable if we take some simple steps to make it so. The light is strong and chemically active, and we can use the "critical" image (the radiant actually focussed on the object) without projecting unsightly markings on the negative.

Two kinds of jets are in use: the "blow-through," or, as it is sometimes called, the "safety" jet, and the "mixing" jet. In the former, coal gas from the house main is led through a comparatively wide tube to the nozzle, where it burns at the ordinary pressure; a stream of oxygen is "blown through" the burning coal gas, and the two gases impinge together on a piece of limestone, or a piece of material partly composed of lime, and known as a "soft lime." The mixing jet is about three to four times as powerful as the blow-through; in the mixing jet both the coal gas—or hydrogen—and the oxygen are forced into the jet at some pressure, mix in a "chamber" near the nozzle, and emerge from the nipple mixed. In short,

we have to do with two varieties of blow-pipe making a piece of lime incandescent over a certain area of its surface. The mixing jet being more powerful, we use with it a lime as hard as we can procure, and best of all is a piece of true limestone, which may be got under the name of "Nottingham" lime. The limes are turned, sometimes very roughly, to the shape of a cylinder, the "lime-pin" of the jet passes up through the middle of the cylinder, and so the lime is kept in position with relation to the nipple of the jet. The mixing type of jet is superior to the other, not only for its greater brilliance, but also because the point of light is smaller and of better actinic quality in the mixing than in the blow-through jet. For the blow-through jet we require gas from the main, and oxygen under some pressure. It is easy to make oxygen gas, and to put it under pressure in a gas-bag, but nowadays this gas can so easily and so cheaply be procured in metal cylinders that probably no one thinks of making his own oxygen. The Brin Oxygen Co. supply oxygen in cylinders; as the gas is under very great pressure a small cylinder holds a large quantity of gas.

For the mixing jet we require both oxygen and hydrogen under pressure, and the hydrogen—or coal-gas—is also supplied in cylinders by the same company. But the light of a mixing jet is so powerful that one can hardly look down the microscope tube without damage to the eyes, unless much of the light is cut off by coloured glasses; and we are able to get the small point of incandescence, with the good quality of the mixing jet, by the following device, still having quite as much power of light as is needed for any ordinary purpose.

A mixing jet is procured, having the nipple considerably smaller than usual, and the hydrogen tube of this is connected with the house gas, while oxygen from a cylinder is conveyed to the oxygen tube of the jet. The only point requiring attention is that the tubes be not allowed to become stopped by dirt of any kind; if such a stoppage were to occur there might be danger of oxygen being forced into the gas supply of the house. We have used this arrangement constantly for years, and have never had the semblance of a mishap. Gas cylinders should always be fitted with some kind of "regulator," for regulating the pressure and amount of gas in the jet; no regulator is better than Beard's Patent, and another finds much favour—the regulator of the Brin's Oxygen Co. It is also a great convenience to have on the cylinder a "pressure gauge"; by means of this we can tell how much gas is in the cylinder at any time. All these fittings for the lime-light are well known, and can be procured from almost any optician.

The following device will not only save much gas, but enable the worker to get a much more equable illuminating power from the lime-light than he would get by the ordinary method of turning up the jet-taps each time that the light is required. To the mixing jet is fitted a device known as a "cut-off," which consists essentially of two extra taps, one for the hydrogen being a partial stop, the other for the oxygen being a complete stop, on the gas-way.

The jets are so made that the light is arranged at its best point with the ordinary taps, the cut-off taps, both worked by one hand-piece, being wide open; when now

the extra, or cut-off, taps are turned down the oxygen is completely turned off, while a small jet of hydrogen continues to burn at the nipple. When the cut-off taps are again turned on the light returns to its original point of brilliance, and so gas is saved, and we have a constant light. Without this device we should either have to let the light burn during the time we are developing a plate, or we should have to guess at the same power of light used for the previous exposures. The cut-off arrangement was originally made by Messrs. Newton, of London, to the writer's design; but can be obtained in other forms from several opticians, Mr. Baker among the number.

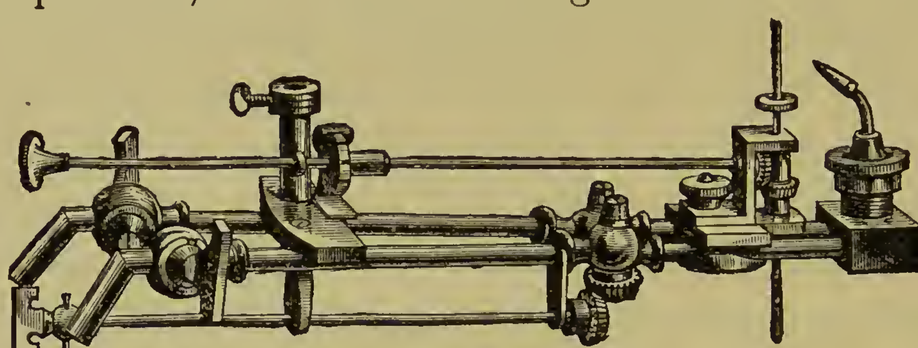


FIG. 20.

In lighting a lime-light burner it is well to turn on first a little hydrogen and light it; then to work up the two gases gradually, first a little H., then a little O , then more H. and more O., till there is at last so much H. that the O. cannot whiten it, and then to turn down the H. till the light is small in area and pure in quality. A red light betokens too much H.; a lurid light means too much O. for the H.

The jet should be supported in such a way that it can be moved both vertically and horizontally, and be firmly clamped when the central position is found, as will be described later.

What is known as the "bull's-eye" may very well be considered as part of the illuminating-system, but we prefer to call it the "paralleliser," as that represents its true function in most cases. It is in its simplest form a plano-convex single lens, with a diameter of about three inches. Being placed between the light and the condenser, the light being in its principal focus, it casts a parallel bundle of rays on the condenser, which has the effect of spreading the light evenly over the field on the ground glass in cases where, without the paralleliser, we should have the field unevenly lighted; but on account of not being corrected in any way, it introduces certain errors into the projected image. Much to be preferred is a "doublet" paralleliser, somewhat similar to a small "Herschel" condenser; and fig. 21 shows a very good form designed by Mr. E. M. Nelson and used by many workers, ourselves among the number.

FIG. 21.

This instrument can also be used as a condenser, *i.e.*, to cause the rays to converge on an object on the stage; in this case it is moved back and forward till the proper position is found for it. This method of using

the "bull's-eye" is for very low power work only; for instance, it works well with the 1in. (aa) of Zeiss, and similar lenses.

The paralleliser (or bull's-eye) of whatever type should be fitted with either a good "Iris" diaphragm, or an opaque disc having a small central aperture; this will be found necessary for centring the paralleliser, and for focussing the condenser to the parallel bundle of rays; the focus for parallel rays being, of course, shorter than for diverging rays. Without some such device as that suggested, we should be unable to adjust this focus, as the parallel rays form no image—of the radiant, for instance—on which we could focus.

The gas-burner, known as the "Welsbach," yields a light of great actinic value, and it may be used for low-power photo-micrography, but it will be found that when the condenser is in focus, the image of the "mantle" will obtrude itself upon the field; this is, to a certain extent, obviated by the use or a paralleliser, but still the light is not much to be recommended.

For the very lowest powers it will be found helpful, if not necessary, to use a piece of ground glass behind the substage; this, combined with a parallel beam from the radiant, will be found to solve a difficulty which often crops up in work with such glasses as the 70 and 35 mm. glasses of Zeiss.

For very low power work it has also been found useful to adopt as condenser, and to place close up to the object, the field glass of a low-power Huyghenian ocular, as suggested by Mr. Nelson.

CHAPTER VII.

Examples of Procedure. General Routine.

IN the actual procedure for making a photo-micrographic negative certain steps must always be taken, some others vary according to the class of work to be performed. In every case the optical system, the lighting and the plate, must be "centred."

1.—To Centre the Condenser.

Affix to the tube in the ordinary way a low-power objective, say 1in., and an ocular. Put the condenser into its place in the substage; insert into the condenser a very small stop, or put on its "nose" a pinhole cap which is supplied with condensers which have no iris. Throw a beam of light on the back of the condenser, and find the image of the small stop in or on the condenser, looking through the ocular and objective; by means of the centring screws of the substage get this stop-image accurately into the centre of the field.

2.—To Centre the Light and Focus the Condenser.

Put an object on the stage, and focus it with the objective; having removed the small stop from the condenser, rack the condenser up or down till, if possible, an image of the radiant is seen sharp across the image of the object. If the radiant-image does not fall on the field the *light is to be moved* till it does; but the condenser

is not to be shifted by the centring screws. When the image of the radiant falls on the object, rack the condenser till, as above, the light-image is seen sharp across the image of the object also sharp. The object is now in the plane of the conjugate foci of objective and condenser, and the image is, so far as focus goes, "critical." The condenser is now centred and focussed.

NOTES.—If an oil lamp is used, the wick should be turned with its edge towards the condenser; for the very lowest-power work the side of the wick may be presented. The image of a wick-flame will appear as a streak of light down the middle of the field, the incandescent area of a lime will appear as a round, or oblong, area; the size of either will depend, *cœteris paribus*, on the distance of the radiant from the condenser, and on the focal length of the condenser. A high-angled condenser, being presumably of short focal length, will give a small image of the radiant, and for low-power work, such as we propose to treat first, it is generally necessary to remove from the condenser its top element, usually hemispherical. In any case the condenser, *as it is to be used*, is to be focussed as described. If the image of the object, as seen through the objective, is "misty," or flooded with light, there is too much angle being used for the objective, and the condenser must be shut down till the image is freed from the mist.

In every case the light and condenser must be centred and the condenser must be focussed; from this point variations in procedure may come in according to the class of work to be done, and the appliances to be used.

3.—The Use of the Paralleliser.

It will be easily understood that for general work the "streak-image" is an inconvenient and unsightly one, and, as a matter of fact, it is found very difficult to get with this image an evenly-lighted field, even when the objective, ocular and stretch of camera combined give sufficient magnification to spread the light fairly well over the field on the sensitive plate. But we have a very simple plan for getting the field evenly lighted, and this plan does not damage the quality of the result, even for the most critical work. The plan is to interpose between the radiant and the condenser a paralleliser or "bull's-eye." The nature of this appliance has been described, and its use is simple if the principles are understood. The light is to be in the principal focus of the paralleliser, and to achieve this we turn the flat, or, with the doublet paralleliser, the concave, side towards the light, and we place the lens at such a distance from the light that a parallel bundle of rays is projected forward from the lens on to the condenser. This position is constant, and the bull's-eye once in its position need not be changed. If a bull's-eye be placed about three inches from the radiant, and a piece of paper held so that the rays pass from the lens to the paper, it will be found that, when the paper is withdrawn from the lens, the disc of light either increases or diminishes in size. If the size increases the light is too near the bull's-eye, if it diminishes and finally disappears the light is too far from the lens. Midway between these points is the true principal focus of the paralleliser, and at that distance it should be fixed. The

paralleliser must also be centred to the radiant, and this position may be found by placing the eye centrally in the rays proceeding from the paralleliser. Fig. 22 shows the appearances seen by the eye in this position when the paralleliser is properly, and improperly, placed with regard to the radiant, E representing the radiant and P the lens; A shows the appearance when the position of the paralleliser is correct, B when the lens is too near the light, C when it is too far away, D when the light is in the focus of the lens, but not central.

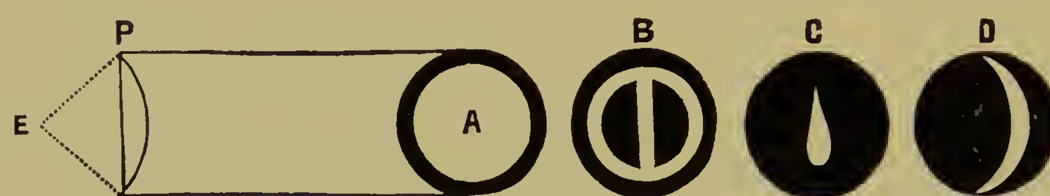


FIG. 22. (NELSON.)

Having now interposed the paralleliser as above directed, but not having moved the light, we proceed to centre and focus the condenser to the parallel beam, the focus being different from that for the diverging beam. To effect this, we place in the paralleliser a small aperture of the iris, or the opaque disc with the aperture described on page 78, and we rack the condenser till we get the image of this aperture sharp on the object, after which, if necessary, we remove the disc, or open the iris. If the previous operations have not been accurately carried out, we may find the disc aperture not in the centre of the field; if there is no great inaccuracy in this respect we may rectify it by means of centring screws for the paralleliser, or, better, by moving light and paralleliser together.

After these dispositions of the paralleliser, the field will be brilliantly and evenly lighted all over; and the writer uses this arrangement almost always, no matter what power he is using either of objective or condenser. Once the light and the bull's-eye are arranged, they need not be moved, and the convenience and certainty of having an even lighting are of great consequence. To get the best effect from the paralleliser, it should not be too near the condenser, eight or ten inches will be suitable; and as the rays are nearly parallel, no notable loss of light-power will be entailed. It is well to have a cell with parallel glass sides, filled with *boiled* distilled water, between paralleliser and condenser; there is no advantage in adding alum to this water.

4.—The Glass, or Cell of Liquid,

used as a "*screen*," or *light-filter*, may be placed anywhere between paralleliser and condenser; in an ordinary way, the glass used need not, for this position, be optically "worked," but if it is to be close to the condenser, or screwed into it, then it should be "worked." And either cell or glass screen, whichever is used, must be at right angles to the optical axis of the whole system.

Having arranged these matters, we are in a position to take steps towards making an exposure. We may take, for an example, an object such as the blowfly's tongue, or a diatom such as a large Aulacodiscus, or, perhaps, a section of a "spine" of an echinus, and our first essay shall be with some low-power objective, as a one inch or two-thirds. The magnification may be about 30 to 35

diameters, and no ocular is to be used. The image is focussed in the microscope in the usual way, notice being taken that the lens is not flooded by the condenser giving too high an angle ; if we look down the tube, having removed the ocular, we shall see whether the entire back combination of the lens is filled with light ; very few objectives—in fact, none except apochromatics—will stand this ; so we shut the condenser till the back of the objective is about half illuminated. We must also make sure that the illumination is even, and the best way to ascertain this is to project the image on to a sheet of white cardboard, when any unevenness will be readily seen and easily corrected by moving the condenser or bull's-eye. But no such movement will be needed if the previous steps have been properly attended to. The microscope is now turned to the axial position for photography, the junction between the tube and the camera is made, and the focussing rod is joined up, if that is the system adopted.—The camera being stretched to the extent requisite for the desired magnification (see page 86), the ground glass is put in its place and the image, roughly focussed, is examined, to make sure that it occupies the proper position on the ground glass. The rough focus may be adjusted by holding a piece of white card in one hand, projecting the image on it, and working the coarse adjustment of the microscope with the other. The plain glass is now put in the place of the ground glass, and the image is focussed with a Ramsden or Aplanat ocular, the ocular previously focussed on the front of the plain glass, and resting lightly on the back of it. By

means of the fine adjustment rod, or pulley, the focus is accurately adjusted, the glass removed, and the dark slide, containing the sensitive plate, put in its place. The flap shutter of the camera is closed, the dark slide opened, and the exposure made. After exposure the slide is closed, withdrawn, and taken to the operating room for development.

The above operations represent generally all that are necessary with whatever objectives we work, but with high powers greater care is needed in matters of accuracy of centring, focussing, etc. When we are dealing with objectives which require correction by collar or by tube-length, earnest attention must be given to these corrections. It is well to use, in examining the object in the microscope, such an ocular as will give about the same magnification as will be given by the ocular and length of camera on the negative ; beyond this all that can be said is that experience, skill, and a knowledge of the object are required to get the best correction.

As examples of *medium power* work we may mention such objects as the fine hairs on the tongue of the blowfly, the dots on a diatom, such as Navicula lyra, or a well-stained preparation of Bacillus anthracis. These may be tried with a $\frac{1}{4}$in. or 6th, using an ocular and at a magnification of 350 to 450 diameters. In the first place, the objective will probably have a correction-collar, which must be so used as to get the best definition, and probably the entire condenser will be used. As before, if the condenser is used too wide open, we may, unless we are using the finest

objectives, "flood" the objective, and, as before, it is well to look down the tube and so arrange the condenser-iris or stop that the back of the objective is about half to two-thirds illuminated. The condenser can, as before, be focussed by using the iris or disc in the paralleliser and a low-power objective; without this it will be impossible to make sure of the condenser being in focus; but some prefer to omit the paralleliser for these powers.

The projection oculars of Zeiss have certain numbers on their shoulder, and the eye-glass is fitted to a small tube which slides in the main tube by a spiral slot. This is to allow of the diaphragm of the ocular being focussed on the plane of the sensitive plate. The image of this diaphragm should be projected on to the ground glass of the camera, and made quite sharp, by moving the eye-glass in its spiral slot. The position of the index on the ocular being once found should be noted, as it will serve for all cases where the same tube-length and camera-stretch are used.

To find the amount of magnification, in diameters, of any combination of objective, ocular and camera-stretch. If objectives and oculars were accurately designated as to their focal lengths there would be no trouble, for a 1in. glass magnifies ten diameters on a plane 10in. behind its posterior principal focus, or, practically, behind its back combination. Then an ocular magnifying three times in diameters would increase the magnification of the 1in. glass at 10in. to 30 diameters; and so on. But as neither objectives nor oculars are generally correctly designated,

we require to use a stage micrometer, ruled to ·01 and ·001in. The image of the divisions is projected upon the ground or plain glass and there measured; the magnification being then easy to determine very closely. With the apochromatics and oculars of Zeiss, a close measurement is obtained by dividing the camera-stretch from shoulder of ocular to sensitive plate × the number of the ocular by the focal length of the objective—all in millimètres. Thus, with a 3 mm. objective, a No. 3 projection ocular and 750 mm. from ocular-collar to plate, $\frac{750 \times 3}{3} = 750$ magnification. But alteration of tube length from 250 mm. throws this calculation out with objectives intended for the 10in. tube.

Suitable tests for the *high* powers will be the secondary markings on some of the finer diatoms; "dots" on Surirella gemma, "dots" on Navicula rhomboides; the flagella on some of the coarser bacteria, as Spirillum serpens, or minute bacteria themselves, as B. Termo, or some Cocci. For these immersion glasses will be used, and the objectives carefully corrected with tube-length or collar. The condenser must also be focussed with even greater care than for the less difficult work. For the finest tests—with diatoms, for example—an immersion condenser should be used, *i.e.*, if the objects are mounted in media of high refraction index. Nothing is gained by the use of an immersion condenser on dry-mounted objects. And for dry objects it is necessary to make sure that the object treated is in contact with the cover-glass, which can be ascertained by the use of a "vertical illuminator," such as is, or used to be, made by Messrs. Beck.

In focussing with the high powers on the plain glass of the camera, the "spectacle-lens" focussing eye-piece of Dr. Bousfield, see p. 41, will be found superior to the Ramsden or Aplanat.

As the exposure increases with the magnification, the greatest care must be exercised to avoid tremor in high power work; and the heat given off by the radiant, be it oil or lime-light, often causes displacement of the parts of the microscope, and consequent blur of the image; it is, therefore, well to let the light burn in its place for some time previous to making an exposure, examining the image just before putting in the plate. In cold weather, this is specially to be observed. Unless the room is specially steady, no walking about in it should be allowed during exposure.

It is better to have the room where this work goes on only dimly lighted; not only is it easier to focus in a dim light, but there is less chance of reflections from the various parts of the apparatus damaging the image on the plate.

CHAPTER VIII.

Photographic Plates. Exposure.

IN view of the observations lately made and noticed on page 51, it may be stated at the outset that, if ordinary achromatic lenses are to be used, it may be taken for granted that orthochromatic plates will always be used. Plates "corrected for colour" are made by two firms in this country, Messrs. B. J. Edwards & Co. and the Britannia Works Co. at Ilford. These firms sell the plates under the name of "Isochromatic." Briefly put, the result of the treatment of the plates or emulsion is that the plates are abnormally sensitive to the yellow region of the spectrum as compared with the violet and blue. Ordinary plates are very much more affected by the violet end of the spectrum than by the yellow region; orthochromatic plates are *relatively* much more sensitive to the yellow. When a "screen" is used, usually a piece of yellow glass, some of the violet and blue rays are cut off, and so the yellow-sensitiveness of the plate is "eked out" by the reduction of the violet and blue rays.

The Isochromatic plates are sold in at least two qualities as regards sensitiveness, the "instantaneous"

and the "medium." The latter are more useful generally for photo-micrography, as with them it is easy to get good density and contrast. But for objects presenting in themselves great contrasts the instantaneous plates may sometimes be preferable.

To render ordinary plates orthochromatic we proceed thus. The plates are bathed in the following solution for two minutes, and then dried in a drying press such as is used by photographers:

Solution of erythrosin (1 in 1,000)	1 part
Dilution of liq. ammon. fort. (1 to 10)	1 "
Water to	10 parts

The erythrosin is the sodium or potassium salt of tetraiodofluorescein, and is made by the Badischer Anilin und Soda Fabrik. In solution it is not fluorescent.

The use of the dyes of the eosin group, of which this is one, with an alkali, is claimed under the Tailfer patent, of which the two firms named above are owners and sole licensees, but by permission the process may be employed for experimental or personal work, so long as no commercial use is made of it.

The dye, cyanin, renders plates sensitive to even red rays, which erythrosin does not, and cyanin may be used; but in this case the work has to be carried on in almost complete darkness, though some of the red light may be cut off by green glass or other medium. Even for yellow sensitive plates great care must be taken to work in a very

subdued ruby light, and not to expose plates more than necessary to that.

Cyanin (1 part to 1,000 absolute alcohol)	...	1 part.
Liq. ammoniæ (1 part to 10 water)	... ...	1 part.
Water to	... ...	10 parts.

Besides the commercial plates already named, very colour-sensitive plates, manufactured by Vogel and others on the Continent, may be obtained from Mr. R. Gotz; some of these, treated with "azaline," are extremely sensitive, and for several purposes useful. And besides the dyes mentioned, others are available—*ex. gr.*, Indophenol and malachite green, but for an account of these the reader must be referred to photographic literature.

With objectives specially "corrected for photography," and with apochromatics, ordinary plates may be used, and in some cases they yield better results than plates specially sensitive to yellow. Here again, when there is difficulty in getting density, a thickly-coated slow plate should be used. The "Sandell" plates of Messrs. Thomas and Co., or the "Thickly-coated Landscape" of the same firm, are good examples of such plates; also the "Ordinary" plates of Messrs. Cadett and Neall. Where there is danger of over-contrast, a plate of the "Portrait" type may be used; these will be found in great numbers on the market.

EXPOSURE.

If we had to deal only with objects presenting so much obstruction to light-rays as could be accounted for by their opacity, it would not be difficult to arrive with almost unfailing precision at the proper exposure for the

various objects to be photographed. Thus, with given illumination and given aperture, the exposure necessary is in direct proportion to the diameters of magnification; and the exposure for one magnification being once determined, all other exposures could be calculated from that one. But when we come to alter the aperture of the condenser, for example, to suit our objective and our object, and specially when we come to deal with thick and thin sections, and still more with different colours, we find ourselves entirely at a loss for any useful rule. Various ingenious devices have been put forward for estimation of exposure, notably that of Dr. Bousfield, who measures more or less accurately the illumination on the ground glass, but none of these devices take really useful account of the colour of the objects. We are in the same uncertain state as to the sensitiveness of our plates, which is, of course, an important factor in the determination of exposure; for none of the standards of plate-sensitiveness allow for colour in the object. Consequently we do not propose to give any rule for exposure, preferring to advise the beginner to make experiments for himself, and to observe carefully the results of his various exposures. We shall also advise that the developing solution, once chosen, shall not be materially altered, and we shall give such details in a later chapter (XI.) as may serve to indicate in what direction the worker may have erred in exposure, by pointing out the appearances and character of negatives when mistakes have been made in this respect.

Reds and yellows in our objects may entail an

enormous increase of exposure; blues and violets are highly actinic, and require little exposure; if the latter colours are pale it will often be found very difficult to get a good strong background without over-exposing the object itself. The yellow screens used with orthochromatic plates may increase the necessary exposure for an ordinary plate twenty or more times, while an orthochromatic plate may require, with the same screen, only two or three times the normal exposure.

If it is seen on development that some mistake has been made in exposure, it is, as a rule, of little use to alter the exposure *slightly*; it is better to double or halve it. Exposures should be varied in geometrical rather than in arithmetical "progression;" if an exposure of 40 seconds is found to be wrong it is better to try 20 or 80, or even 10 or 100 rather than 35 or 50. For some reason we find that if we get a poor negative the remedy, in 90 per cent. of the cases, is to double the exposure.

If the background of a negative is weak we have to look to one of three causes: 1st, under-exposure; 2nd, reflected light inside the apparatus; 3rd, gross over-exposure, but in this case the whole image is weak and grey. Under this head may fall the effect of flooding the lens with too much aperture, but this is seen in the microscope; and under-development, which is also easy to discover and to avoid in future.

CHAPTER IX.

Colour-treatment of Various Objects.

WHEN we come to classify objects in regard to their adaptability for photographic treatment, we find two prominent characteristics, each presenting a difficulty: 1st, want of colour or contrast; 2nd, actual or actinic opacity and violence of contrast between object and background or between various parts of the object.

Under the first heading come a large number of objects pellucid, unstained or lightly stained; also objects stained unsuitably or faded.

Pellucid objects give the photo-micrographer much trouble, especially when the substance composing them is of high refracting index. Diatoms having a siliceous framework are not much trouble in this respect till we get to very high aperture, and then an optical difficulty crops up of a nature too abstruse to enter into here. Starches, however, unstained yeast, and the like are extremely difficult to manage, for it is a *crux* to get contrast between object and ground; a very useful plan, sometimes the only available plan, is to stop out the central pencil of light by putting into the condenser one of the central opaque discs shown in fig. 14. There is a great temptation in dealing with this kind of object to shut down the condenser to such a point that there appears to be contrast; but this leads to fuzzy diffraction images, and should, so far as possible, be avoided.

With pellucid and very lightly stained objects the contrast will always be increased by stopping down the condenser, but in so doing we are impairing definition, and we should make a point of stopping down just as little as possible. But it is admitted that sometimes a considerable amount of aperture must be cut off to obtain anything like contrast. The central stop is decidedly the best help in such conditions.

If the object is slightly stained with any colour whatever we have to meet the difficulty by use of colour-correct plates helped out with a screen. Broadly, the line to follow is to use a plate little sensitive to the stain colour, and to use a screen cutting off the colour of the preparation. Thus, if the weak stain is violet, as logwood, or pale blue, as methyl blue, our plan would be to use a yellow-sensitive plate and a strong yellow screen. If the stain is weak yellow, as Bismarck brown, or red as some eosins, we may use an ordinary plate, with an apochromatic or corrected lens, or an orthochromatic—erythrosin—plate, in either case with a dark blue or green screen. Some of the eosin stains are very deceptive; they appear red or pink to the eye, but are found to transmit much blue. For these we find a green screen with an erythrosin plate to answer well. With pale stained, or faded, objects contrast may to a certain extent be secured by stopping down, but, as before, it is always at the expense of definition. Under this head come a great number of preparations made by those who are not aware of the *desiderata* for photography; we have sections stained poorly with violet logwood, bacteria with

weak gentian or even methyl violet, and with over-washed methyl blue. When we have any of these dyes properly used there is little trouble; and in our Appendix attention will be called to this matter of staining suitably for photography. The principle to be borne in mind is, to use a plate comparatively insensitive to the stain-colour, and a screen cutting off more or less of that colour.

On the other hand, when we have to treat objects of great density, or heavily stained with some non-actinic dye, we must follow other courses. The best example of this kind of object is that of the usual insect-preparations, and of thick sections which are all too common. The treatment of such objects consists chiefly of prolonged exposure, and the omission of everything which will cut off rays of the colour of the object, as well as the use of plates highly sensitive to the colour. Thus a very yellow insect may be tried with a yellow-sensitive plate, without any screen; a thick logwood preparation may be tackled with an ordinary plate, or, if the lens is achromatic, an orthochromatic plate, possibly with a blue screen to keep back the ground till the object is impressed on the plate. In practice, however, prolonged exposure alone usually gives the best available result with this class of object.

When we come to an object partly dense and non-actinic, and partly weak and highly actinic, then, indeed, a difficult task is before us. Marked examples of this class are insects with deep yellow bodies and delicate pale legs; sections with bacteria stained blue and tissue red, or even the bacteria red and tissue pale blue; preparations stained

briskly with haematoxylin and counter-stained with weak eosin or orange; colourless bodies in strongly-coloured media, and the like. Here is room for display of skill. For such cases the principle to follow is to cut off the pale or non-actinic colours so that they may not be impressed on the plate till the other details have had time to impress themselves. (At the same time it is to be noted that a screen, cutting off actinism from the weak parts, is also doing the same from the actinically dense parts, which increases our difficulty.) As instances, Mr. Carnell, who has been singularly successful with insect-preparations, uses for them yellow-sensitive plates, a yellow—oil lamp—light, and a dense yellow screen to boot, giving, of course, great exposure. If we have ever really succeeded with blue bacteria in red tissue it has been by using a dense yellow screen and a plate extra blind to blue. To keep back pale eosin in a logwood-stained (nuclear) preparation we have used as a screen the solution of Zettnow:

Copper nitrate	160 parts.
Chromic acid	14 ,,
Water to	250 ,,

A thickness of this of about one centimètre passes only yellow-green rays. In practice it will be found more convenient to use pieces of glass of suitable colour; a selection may be made from the odd bits of any glass maker, as, for example, Messrs. Cookson and White, London. Solutions of aurantia, turmeric, chromic acid, tropœolin, etc., have been used and may be tried. For blue screens solutions of ammonia-sulphate of copper may be used; but

again, for all practical purposes, glass will be found equally good; a few samples of cobalt blue and "signal-green" glasses of various depths may be obtained.

The more actinic our light is the more pronounced must be our screens. Thus, if we use an oil lamp, we do not require so strong a yellow screen as if we were using lime-light; sunlight would require very strong screens whether blue, green, or yellow.

It is impossible to formulate instructions likely to be useful for all circumstances that meet the photo-micrographer; the plan is to master the principles governing the sensitiveness of plates, to learn to appreciate the colours of objects, and then, by experiment and practice, to arrive at the proper methods of procedure for the various cases that present themselves. Properly-cut and stained preparations will be found to present no real difficulties; for this reason we shall devote attention to the subjects of cutting and staining in a later chapter.

CHAPTER X.

Black Backgrounds. Reflectors. The Lieberkühn. The Polariser.

THE illumination known as "black background" is by no means so much appreciated or used as in our opinion it deserves to be. Not only is this illumination a fine test for the qualities of objectives, as pointed out by Mr. Nelson, but there are not a few subjects besides diatoms which are shown in a highly beautiful and valuable manner by this treatment. We find its advantage when dealing with physiological and other objects stained by one of the "metallic" methods—for instance, "endothelial" tissues, such as mesentery stained with silver nitrate; nerves, and Pacini's bodies with gold; and muscular and nervous tissues mounted *au naturel;* sections of unsoftened bone, and various other objects. Many low forms of animal life and microscopical plants may also be photographed on a black ground. It will, however, be found difficult to treat in this way tissues stained in the ordinary styles, especially if they are multiple-stained; but in such a case we cannot expect to see more with a black background than by the usual method.

The best method for obtaining this result is to use one of the opaque-centre stops (fig. No. 14), and the line to follow is to use the *smallest* of these that will give the object well lighted and clearly defined with the background

quite dark. It will be found difficult to work this system with the high-medium powers, but we are told that even the 3 mm. apochromatic glass has been so used. If with any objective it is found that the dark ground cannot be obtained by the procedure here given, the plan to follow will be to fix into the back of the objective a stop, cutting off part of the effective area of the back combination, or an iris may be fitted to several objectives. Thus we failed with the 12 mm. apochromatic to get the black ground, but on procuring an iris stop for the back of the objective, as suggested, we overcame the trouble.

The method of procedure is as follows: With one of the lower powers, as one inch, or a higher power with the stop, focus objective and condenser, using the paralleliser as usual; then insert into the condenser the smallest "spot" stop. By racking the condenser, try to get the black ground; if this does not succeed, put in the next larger stop, and so on till the object is sharply and brightly lighted, and the ground quite black. The exposure will, of course, require to be long, and development will be puzzling till experience has been gained. It is a mistake to develop to great density; and, of course, the background must not be allowed to show development to any marked extent. *Slight* "greying" under development will "fix out."

Illumination by Reflected Light.

Objects are sometimes of such a nature, or mounted in such a manner, that they must be examined, and photographed, by reflected light; and frequently photographs of such objects are both beautiful and valuable.

At various times various instruments have been used for this kind of illumination, but we need refer to only a few, as the others have been superseded by later and better appliances.

It is assumed that the objects are either mounted on some opaque ground, or have some opaque material placed behind the ordinary mount. The first method that will suggest itself is to use some low angle condensing arrangement, such as a bull's-eye, above the stage. This may be done. The plain side of the bull's-eye is presented to the object, and the light and the bull's-eye are so arranged that the light is brought to focus on the front of the object. The light and bull's-eye should be placed as near to the tube of the microscope as convenient,

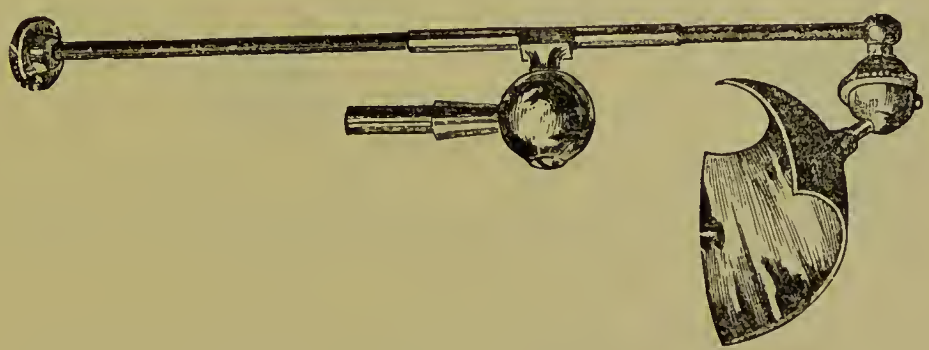


FIG. 23.

and it will be seen that this system is available only for low powers where there is plenty of room between the front of the objective and the object. If the direction of illumination is at too wide an angle with the optical axis, much of the light will be totally lost by reflection.

The next, and a better, method consists in the use of a "side reflector," such as is made by many opticians An example is shown at fig. 23.

This appliance should not be attached to any moving part of the stand, but to the stage or the limb of the stand. The reflector should remain steady, all but touching the preparation, and right opposite it, in a line at right angles to the optical axis, is placed the light, the rays from which should be directed in a parallel bundle through a bull's-eye right on to the reflector; from it the rays are reflected down upon the object, which is thus brilliantly illuminated. For this work also the exposure must be long.

The reflecting device known as a "Lieberkühn" is both very pretty as a scientific instrument and very valuable

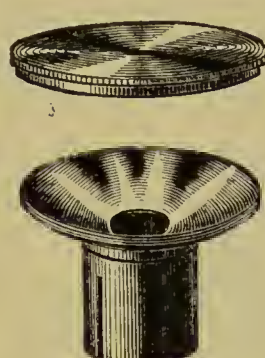


FIG. 24.

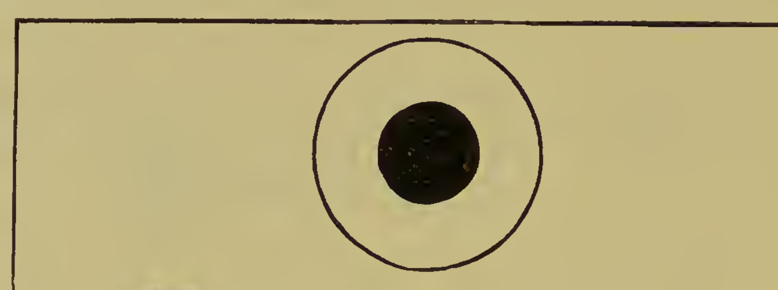


FIG. 25.

in use, but it necessitates a special mount, or a simple enough addition to an ordinary mount. The light is passed up from below in a parallel bundle *round* the object, falls on the polished front of the "Lieberkühn," and is reflected down from the latter on to the object. Fig. 24 shows the instrument, and fig. 25 shows the method of mounting objects for its use. The central part of the mount is blocked out, and this may be done in the mounting, or by the microscopist. A piece of court-plaster of suitable size may be cut out and fixed

to the back of the glass slip behind the object, or another slip bearing the opaque spot may be placed under the object to be photographed. The chief objection to this appliance is that we require a "Lieberkühn" for each focal length of objective used; otherwise we shall not get proper illumination. The exposure here is not so protracted as with the two last illuminators.

The "Lieberkühn" fits rather loosely on to the objective, and, after the object is in focus, the "Lieberkühn" is moved up and down till the brightest and most even illumination is found.

We give no attention to "spot lenses," "paraboloids," and the like, believing them to be wholly inferior to the instruments we have noticed.

Very useful photographs of microscopic objects have been produced by polarised light. A polariser and analyser of the usual kind are employed, choice being made of prisms as large as may be obtained, and in most cases very prolonged exposure will be required.

CHAPTER XI.

Development of the Plate. Fixing.

NOT wishing to create confusion in the mind of the beginner, we shall confine attention to three developing agents; these varying in their characteristics only slightly, but still to such an extent that it may be said that one developer is on the whole more suitable for a given kind of work than another.

Hydroquinone, or *Quinol*, is our favourite for general work, especially where we wish good contrast between object and background.

Pyrogallol falls little short, if at all, of quinol for all-round work; but contrast is not so much a characteristic of the former as of the latter.

Metol-Hauff is a developer using which we may give short exposures, and get considerable contrast; for objects presenting in themselves considerable contrasts this will be found an excellent reagent.

The Pyrogallol Developer.

Dissolve 4 ozs. *avoir.* of pure sodium sulphite in about 8 ozs. of distilled water, when dissolved add 120 grains of citric acid, and pour into a commercial ounce bottle of pyrogallol, make up to 9 ozs. Filter and label "Pyro, 10 per cent."

Take 1 oz.—fluid—of liq. ammoniæ, sp. gr. ·880, and add 9 ozs. of distilled water. Label "Ammonia, 10 per cent."

Note.—When a phial of liq. amm. fort. is opened it is well to dilute it at once with equal measure of water, or till it shows on the hydrometer sp. gr. ·920. This, of course, is half strength, and double the quantity of it must be used as compared with sp. gr. ·880.

Take also 1 oz.—chemical—of potassium bromide, and dissolve it in such a quantity of water as will, when solution is complete, make the whole amount to 10 ozs. fluid.

A normal developing solution may consist of 20 mins. of pyro solution, 30 of ammonia dilution, and 10 of bromide solution. (The developer thus consists of one grain of bromide, two of pyro, and three minims of strong ammonia in an ounce of water.) The solutions separately will keep for a long time in good order; the ammonia should of course be kept in a well-stoppered bottle; the solutions once mixed will not keep good for many minutes. The pyro solution may be obtained made up from any photographic dealer under the name of "Sulpho-pyro-gallol."

If it is found inconvenient to measure so small a quantity as 10 mins. of the bromide, a 5 per cent. or any other convenient solution may be used as "stock."

When a plate has been nearly properly exposed and this developer is applied, details may be expected to begin to appear in about twenty to thirty seconds; the details will creep up gradually, and density will increase gradually till no further action appears to take place. If experience tells us that the development has not at this juncture gone far enough we add, say, 10 mins. more of the ammonia solu-

tion. When development is complete the plate is well washed and placed in the fixing solution.

In place of ammonia, one or more of the *alkaline carbonates* may be used as the alkali, and many workers prefer the carbonates. Bromide, in the case of such a developer, may be omitted, or present in minute quantity, the following representing a good average developing solution:—

Pyrogallol (30 mins. of the stock solution with sulphite given above, or)	3 grains.
Carbonate of potash or soda, or both, in equal parts	16 ,,
Potass. bromide (*nil*, or)	½ ,,
Water to	1 ounce.

This will develop a plate rather more slowly than pyro-ammonia; it is little apt to produce green fog, and good contrast and density can be obtained. The film is likely to be stained yellow, but the acid alum bath (page 111) will remove the stain in a short time.

If the details rush up quickly under the pyro-ammonia developer, and if density does not increase at a rate commensurate with the details, the plate has been over-exposed, and we may add bromide to the developer; this will check the detail considerably, the density less. If, on the other hand, neither detail nor density come up readily, we may add ammonia; if density comes in some parts, and the other parts lack both detail and density, then we add water to the developer, and then ammonia. (This reduces the proportions of bromide and pyro, and increases that of the alkali.) Broadly speaking, pyro gives density, alkali gives detail, and bromide restrains the action of both.

The Hydrokinone Developer.

A.	Take hydrokinone	80 grains.
	Sodium sulphite	1 ounce.
	Citric acid	30 grains.
	Potass. bromide	15 ,,
	Water to	10 ounces.
B.	Take potass. hydrate—"caustic potash" ...	80 grains.
	Water, distilled and boiled	10 ounces.

These solutions may be obtained from Messrs. Thomas, of Pall Mall, London, to whom the formula is due.

A suitable developer will be found to consist of one part of A, one of B, and one of water.

With this developer the details will not come up so quickly as with the last, but the action will not die away so soon; and one dose of this developer may be used at least four times, *i.e.*, for four plates. If after two are developed the action seems to become slower, a small addition of the alkali may be made. And we generally add less than one part of water; thus, we take half an ounce of A, the same of B, and we make up with water to 10 drams only instead of 12. It may be found difficult to keep the caustic alkali in solution energetic for any length of time, as it becomes carbonated; the plan is to boil the water for solution, and to keep the solution in several small bottles full to the top and well stoppered. And we buy the alkali in small quantities, as it keeps badly even in the solid state.

The best results seem to be got with this developer when it takes about ten minutes to fully develop a plate; the contrast is then admirable, and if exposure has been sufficient the details are fully reproduced.

The Metol Developer.

Metol-Hauff is a "proprietary" reagent, and may be obtained from photographic chemists under the above name.

A.	Dissolve metol	1 part, or 50 grs.
	in water	100 parts, or 10 ozs.
	Add sodium sulphite	10 parts, or 1 oz.
B.	Potass. carbonate	1 part, or 1 oz.
	Water to	10 parts, or 10 ozs.
C.	Potass. bromide	·5 part, or ½ oz.
	Water to	10 parts, or 10 ozs.

Normal Developer:

A 1 part.
B 3 parts.

To each ounce of these mixed add 20 mins. of C.

This solution of metol (A.) keeps good for any time, and even the complete developing solution will keep for a long time, and may be used several times. Under this developer the image comes up much more rapidly than with the others. In fact, the beginner may be alarmed on first using this developer; but, if time be given, due density will come, provided the plate is not greatly over or under-exposed, and the plate will hardly ever fog with metol. Where there is no great difficulty in getting contrast, or where great contrast is not required, this reagent should certainly be tried carefully; if the developer is diluted the contrast is lessened; bromide slows the action. Development may be started with a mere trace of alkali, and detail and density will still come *after a time*. Or we may develop in a "tentative" way; beginning with little alkali, and when the image is faintly seen in all parts, we may complete development by the addition of alkali

quant. suff. The fingers should not be immersed in this developer more than necessary. It is apt to produce a skin disease with some persons.

The *manipulations of development* are simple. The plate is removed from the dark slide, and it is useful now to write on the film, with a pencil, any details which may be required as memoranda; the magnification, the number or name of the preparation, etc. The greatest care must be taken that the light is "safe," and the dish in which the development is carried on should be kept covered as much as possible during the operation. But when once the image has come well out on the plate the danger of fog from unsafe light is considerably less than before development. The plate is laid film upwards in the developing dish, and the solution is swept over the film, so that all parts of the film are wetted at once, or as nearly so as possible. The plate should be kept rocking, more or less, during the operation; if this is neglected there is danger of uneven markings appearing on the plate.

Experience alone will teach when to stop development; some plates require to be carried further than others; some subjects require the negatives to be made denser than others. But when the development is complete, the image, looked *through*, appears *very much too dense.* Details may or may not show on the back of the plates; as a rule they do show. It is always safe to err on the side of full development; for if the plate is too dense it is easy to "reduce" the density as explained on page 115. But there are a few occasions, as, for instance, when a preparation is much wanting in contrast of actinicity—as

poorly stained or bacteria preparations—when it will be better not to over-develop, but rather to get detail without much density, and without reduction of silver in the image of the critical bodies themselves, and to trust to further operations, such as intensification (page 116).

When a plate is fully developed the image looked *at* is practically a black or very dark grey expanse of film with little more than a suggestion of differentiation in the details. The plate is then washed for a minute under the rose tap, and placed directly in the fixing bath. If, perchance, there are symptoms of the film "frilling," or puckering at the edges, the plate may be put before fixing into a bath of water saturated with common alum. Few plates frill now-a-days, and any that do should be rejected.

The Fixing Bath.

Take sodium hyposulphite 1 part by weight, and water 5 parts by measure. It is quite safe to keep a "saturated" solution of "hypo" in water, stirring the solution before pouring it out into the dish, and adding a small proportion of water. Provided the fixation is complete, and that the film does not suffer from over-concentration of the solution, the exact strength does not much matter. But the solution must not be too weak, nor at all acid. (*Note.*—Fixing baths made deliberately acid have been recommended by good authorities, but they entail extra trouble without extra advantages, so far as we can discover.) A freshly-made hypo solution is apt to damage the film, under certain conditions; the chief reason being the degree of cold produced at the time of solution.

If the hypo bath is found acid, any alkali may be added till the bath is slightly alkaline.

Soon after the plate is put into the fixing solution the grey, unreduced silver bromide will disappear from the film ; when it has disappeared the plate may, for safety, be taken as half-fixed. Many negatives are lost by stopping this fixing operation too soon. When the plate is fixed, daylight may be admitted. It is necessary to remove thoroughly from the film every trace of hypo, and this can only be accomplished by frequent or constant changes of water. A rose tap will wash a plate more quickly than any "washing machine" ever invented. But if water is allowed to run for a few hours into a dish or box containing the plates, they may be considered free from hypo. Ten minutes under a good rose will effect the same. If we propose to intensify later, extra care must be taken in the washing.

After pyro development, the plates are often stained to a yellow colour, which is sometimes useful. But as a rule it is well to remove this stain as follows: To a pint of saturated solution of common alum add two drams of ordinary hydrochloric or sulphuric acid, and immerse the stained plate in this till the stain is removed—a few minutes. Then wash well.

Plates are dried in "drying racks," which are well known in photography. We prefer these racks to have very wide grooves, so that we can let the plates lean slightly over, face downwards ; any dust falling then lights on the back rather than on front of the plates.

If a negative is valuable, or if we expect to take many

prints from it, we should varnish it with one of the varnishes used by photographers. The coat of varnish should not be thicker than necessary, as a thick coat would prevent good contact between plate and paper or lantern-slide plate in "contact-printing."

CHAPTER XII.

Appearances of a Plate during Development and after Fixation. Reduction. Intensification.

IF we carefully notice and properly understand the various appearances presented by a plate undergoing development, we shall have the best of all clues as to whether the plate has been properly exposed, or, if not, in what direction error has been made. And if we fail to apprehend the true state of matters during development, we may arrive at a sound conclusion after the plate is fixed, when we can examine it in good light.

As stated in last chapter, the metol developer causes the image almost to flash up immediately after application ; the pyrogallol developer acts less rapidly, and the hydrokinone considerably more slowly than the pyro, but when we have arrived at the normal rapidity of action with each of these developers, we are in a position to judge whether we have erred in exposure, and, if so, in what direction.

If the image comes up too soon, and is grey all over almost at the first, we may know that we have over-exposed.

If there is reluctance on the part of the image to appear, and if the high lights appear long before the half-tones, and if the high lights become fairly black before the shadows appear, or have any strength, we may be sure that our exposure has been insufficient.

With hydrokinone especially, if the image comes up somewhat rapidly and soon becomes dense *black* all over, we have over-exposed, but not so much as when the image comes up quickly and *grey* all over.

Gross under-exposure leads to no image at all, or very thin images refusing to take density. Considerable under-exposure leads to slowly-developed black and white images. Gross over-exposure leads to a flashing up of the image, greyness all over, and refusal to take density. Slight over-exposure gives generally, but not always, very dense images, wanting in contrast between light and shadows.

After proper exposure the image begins to appear at the normal time, the high lights first, the half-tones closely following, the shadows last; the processes of light and shade development go on gradually, never stopping, never rushing; detail is gained first, and density gradually follows the detail.

It is more difficult to describe a perfect negative as seen after all the operations have been gone through. The background is dense, so that, while the details are being impressed in the operation of printing, the ground shall remain white. The dark parts of the image are fairly dense, yet full of detail, and there is a distinct difference between these dark parts (high lights) and the background. The light parts of the negative—dense or non-actinic parts of the object—are clear, without muddy or foggy appearance, yet full of detail, if detail existed in the object. The gradation from light to dark is even, and a long scale of densities is represented on the negative, unless the object consisted of simply opaque and trans-

parent tissues, as in the case of diatoms. It is practically impossible for a beginner properly to estimate the quality of a negative. It is advisable for him to show his negatives to someone of experience who may point out their shortcomings.

If the gradations of the negative are full, and all the details of the object represented on the plate, but the whole density too great, such a state, in fact, as may arise from slight over-exposure or over-development, it is easy to rectify matters by "reduction." If all the details are represented, but there is a want of contrast in density between one part and another—if the ground is grey instead of black, as may happen after over-exposure or under-development—"intensification" may supply a remedy. But if the plate is dense at one part, and thin and wanting in detail in another, due to under-exposure, it is practically impossible to amend it, and the only plan is to make another exposure. In any of these cases it is, as a rule, preferable to make another exposure; but under certain conditions already alluded to on page 95 the only course may be to intensify with or without previous slight reduction. Thus, when we are dealing with very pellucid, or weakly-stained objects, or such as are difficult to reproduce with good contrasts, the plan most useful is to reduce slightly, so as to remove any trace of fog from the shadows or non-actinic parts of the image, and then to intensify.

To Reduce the Density.

Soak the plate for a few moments in a fresh solution of sodium hyposulphite, such as is used for fixing, and

then in a cup or measure mix with the hypo a few drops of a weak, say 5 per cent., solution of potassium ferri-cyanide, or ferridcyanide—not ferrocyanide. This mixture applied to the plate, and kept moving over it, will in a short time reduce the density, clearing up the image all over, but specially the thin parts. As soon as the desired action has taken place the negative is well washed, as after the fixing bath. If, in order to get a considerable increase of contrast, we propose to intensify after reducing, the washing must be thorough, and the plate, after washing, should be immersed for some minutes in the acid alum bath mentioned on page 111.

To Intensify the Negative.

Make a saturated—at ordinary temperature—solution in water of mercury perchloride (corrosive sublimate), to each pint of which add a dram of hydrochloric acid. Soak the plate in this till the film is greyish white all over and through to the back, and wash well. Prepare a weak dilution of liq. ammoniæ, just smelling distinctly of the ammonia, and immerse the plate in this till the image is black or dark brown right through to the back of the film, then wash. If any stain appears in this operation the plate has not been thoroughly freed from hypo. In place of the ammonia a weak solution of sodium sulphite may be used; this is safer as regards staining, but does not give so strong intensification as the ammonia. If, after either ammonia or sulphite, a scum appear on the plate, it may be removed by rubbing with a small pad of cotton-wool. It is most important to let the second solution act till

the action has *gone right in through* the film; if this is neglected there is grave danger of the negative going wrong after a time. In place of either ammonia or sulphite alone to follow the mercury, one part of the hydrokinone stock solution (A), page 107, to two or three parts of water may be used. This will give a dense, ruddy image, printing with great contrast.

It is not necessary to reduce before intensifying, but the two operations afford the best means known to us of achieving the very difficult task of getting good contrast out of weak objects. If one intensification is not enough we may repeat the operation, taking care that the washing between the operations is effective.

Note.—When negatives are to be used for reproduction by one of the photo-mechanical processes, as for book or article illustration, they ought to be "plucky," *i.e.*, they ought to have clear shadows, and fairly dense high lights; for the production of such negatives, when the objects are devoid of actual or actinic contrast, the procedure of slight reduction, followed by intensification, will yield the best results likely to be obtained. At the same time, when the "markings" or details of an object are extremely minute or fine, there is danger of their being, to a certain extent, blocked or "smudged" by intensification, especially if reduction is omitted.

CHAPTER XIII.

Printing on Gelatino-chloride Paper.

IN order to bring out to the best advantage all the details that may be present in a negative, it is advisable to use a printing process in which the image is as much as possible kept on the surface of the paper, and not allowed to sink into the paper itself. The platinotype process, which is perhaps the most beautiful of all printing processes for ordinary artistic photography, is not so well suited to our purpose as such processes as "bromide paper" or "gelatino-chloride," where the image is confined to a stratum of gelatine on the surface of the paper. The old albumen process is omitted from this book, because the gelatino-chloride process is both handier and better, while the general procedure is the same. But for some subjects, where fineness of detail and absolute sharpness of definition are not essential, the platinotype process will be found speedy and exquisite in its results. For the albumen and platinotype processes we must refer the reader to photographic literature.

Gelatino-chloride Papers

are sold under various names, such as "Aristotype"; but we may take as probably the best, and as representing the others, the "Solio" paper of the Eastman Photographic Materials Co., though doubtless other papers of the same nature may work equally well in some hands.

The paper, previously surfaced with an insoluble and impermeable layer of a barium compound, is coated with an emulsion of silver chloride in gelatine; when the emulsion is exposed to daylight it darkens; the colour at this stage is unsightly, and the image requires to be "fixed." The most rapid way to finish the operations is to "tone" and "fix" in one bath, and this is easily accomplished; but the colour is likely to be of a somewhat warm kind unless the sulphocyanide bath is used, which we consider inferior in most respects to the baths we shall recommend. But we may obtain good black tones, which are probably preferable for our particular work, by the use of other toning baths which shall be given.

This is a "printing-out" process, *i.e.*, the paper and the negative are exposed to daylight, and the image becomes plainly visible on the paper. It is also a "contact" process, because the face of the paper is placed in contact with the face of the negative for printing. The only apparatus required is a "printing frame," well known in ordinary photography; the back is hinged, and so made that one half of the back can be turned away from the paper, and one half of the print can be examined without disturbing the position of the other half in its relation to the negative. The printing frame holding the negative and the paper is exposed to daylight, and from time to time one half of the back is turned aside and the print examined; a little practice will enable the worker to judge accurately when to stop the printing. These papers being rather sensitive to light, the image should be examined as quickly as con-

venient, and that in a subdued light, certainly not in sunshine. If the high lights become degraded by over-exposure to strong light, the bleaching action of the toning bath will not suffice to remove the effect, even if slight, of the improper exposure to light. In all cases the printing is to carried *beyond* the effect desired in the finished print; for there is always a certain amount of bleaching action in later operations; this extra darkening need not be great when the combined toning and fixing bath is to be used; but it must be considerable when we are to use the acetate and gold bath, or the platinum bath. Thin negatives, wanting in contrast, should be printed in subdued, diffused light, strong or plucky negatives in a bright light, hard ones in direct sunlight, care being taken that they do not get over-printed. If a negative is very hard, with chalky high lights and thinnish shadows, it is a good plan to let light act for a few seconds on the whole print after it has been removed from the frame; this will subdue the glaring whites of the print.

Combined Toning and Fixing Bath.

The prints are placed in this bath without any washing after they are removed from the frame. Prints will keep for a considerable time after removal from the frames, if light is kept from them; and the paper will keep a long time before printing in a place *free from damp*.

The following are the Eastman Co.'s solutions:—

Stock Solution No. 1.

Sodium hyposulphite	6 ounces.
Potash alum	1½ ,,
Sodium sulphate	4 ,,
Water, preferably distilled, to	60 ,,

STOCK SOLUTION No. 2.

Gold chloride	15 grains.
Lead acetate	50 ,,
Water, as above	$7\frac{1}{2}$ ounces.

THE BATH CONSISTS OF

Stock solution No. 1	8 ounces.
,, No. 2	1 ,,

Notes.—Gold chloride is sold as "chloride of gold and soda" in 15 grain tubes, hermetically sealed; nick a tube with a file, and break it over the given quantity of water. When the lead salt is added to the gold there will be an orange-coloured precipitate, which will be dissolved when added to the solution No. 1. A precipitate will take place in No. 1, but may be neglected.

When prints are first placed in this bath they take a very ugly colour, which soon gives place to a very good tone; the toning and fixing should occupy about twelve minutes at the least; it is hardly possible to over-tone in this bath. If too short time is allowed there is danger of the fixation being imperfect, which would lead to subsequent fading or yellowing of the prints. Over-toning, if it occurs, is demonstrated by a poor blue tone. When the prints have been about ten minutes in the bath, and show a bluish tone in the high lights by transmitted light, they are to be put into running water and washed till thoroughly free from hypo and the other salts. Washing may be accelerated by laying the prints face down on a slab of glass or ebonite, and passing repeatedly over their back a "squeegee," while the water from a rose tap is playing on them; this should never be omitted. After this treatment the prints will be washed by an hour's sojourn in running

water. If allowed to dry naturally, the prints will have a fairly glossy surface; if a high gloss is desired—and for our work it is often most valuable—they should be squeegeed down gently to a glass or "ferrotype" plate, previously cleaned and polished with a solution of spermaceti wax 120 grains in benzine 2 ozs. The wax solution is rubbed all over the plate, and then, with a dry cloth, *apparently* rubbed all off again. The method of procedure is as follows: Lay the plate face upwards in a dish of clean water, the print face downwards in the same water, bring the two up together, excluding air-bubbles; lay the two down, paper upwards; over the paper lay a piece of indiarubber or American cloth, cloth side downwards, and pass the squeegee briskly, but not heavily, over the cloth in both directions. Let the print dry gradually, and finally strip it from the plate. To give a print strength, if desired, it may be backed while wet and on the plate with a piece of very thin card, the print being backed with thin glue or starch.

The Acetate Bath.

Having printed somewhat more deeply than for the previous bath, and having washed the prints for a few minutes in several changes of water, immerse them in the following:—

Sodium acetate	150 grains.
Gold	5 ,,
Water	40 ounces.

This will give brown tones; and the prints must be fixed in a bath:—

Sodium hyposulphite	4 ounces.
Water	20 ,,

For at least fifteen minutes.

To Obtain Black Tones.

Tone lightly first in the acetate bath, and then immerse in the combined bath till the black tone is obtained, then fix as above in hypo. In every case the prints must be kept moving in these baths; if, at any time during these operations, two prints are allowed to stick together, markings are likely to occur.

Several other baths might be suggested, but those given appear to us the most worthy of attention. By squeegeeing a print to finely-ground glass previously waxed we get a very good mat surface.

Note.—If in the combined bath the prints take the desired tone in less than 12 or 15 minutes, it is well to give them a further bath of hypo for a few minutes to ensure thorough fixation. If it is intended to mount prints on card, retaining the high gloss, the prints, while wet and adhering to the glass plate, should be backed with a piece of backing paper, which is impervious to water. This paper may be caused to adhere by a starch mountant, or, better, by a thin well-filtered solution of glue. This solution is used to cause the backing paper to adhere first to the print, and later to the permanent mount.

CHAPTER XIV.

Printing on Bromide Paper.

THIS is probably the best printing process for the photo-micrographer, but a certain amount of practice is required before good and even results can be expected. The general procedure is this: The paper is exposed to light, generally artificial, in contact with the negative, and the image is *developed* with ferrous oxalate or other suitable developer. The bromide paper consists of very pure paper coated with an emulsion of gelatino-bromide of silver of much less sensitiveness than an emulsion used for negative-making; we may use a fairly bright yellow light during development, but the window or lamp should be provided with yellow fabric or paper; ordinary yellow glass is unsafe. The paper known as "canary medium" answers well, or two layers of "golden fabric."

Paper should be used having on it a *slow* emulsion; the bromide papers frequently found on the market are too sensitive for the best results; the paper recommended is the "Permanent" of the Eastman Co. The tone to be aimed at should be good black, similar to that of platinotype, and with the permanent bromide paper this tone is easily obtained. A paper lately introduced by the Eastman Co. bids fair to replace all other papers for our special purpose; it is called "Nikko."

The first requisite is a standard light; this may take

the shape of a "regulator" gas-burner such as sold by Sugg and others, a "standard" candle, a lamp having its flame always the same height, or an amyl lamp such as is used for certain photometric work. It really does not much matter what light is used provided we get always the same brilliancy and actinicity. Those who have gas at their disposal will do well to use a regulator burner; it is simple, convenient, and sufficiently equable.

A standard distance is also required; the printing-frame holding the negative and the sensitive paper are held at the standard distance from the light. Eighteen inches will be found a convenient standard of distance, but twelve may be taken It must be remembered that the distance must be kept with fair accuracy, because the power of the light varies inversely as the square of the distance from light to sensitive surface; a small mistake in distance may amount to a serious mistake in exposure.

The exposure varies with the density of the negative, other things being equal, taking for granted that we in each case wish a good black and white print. A dense negative requires more exposure than a thin one, and there is great difference between one negative and another in this respect. An average negative may require about 15 seconds' exposure at 18 inches from a gas-burner with Eastman's permanent paper, a dense negative will yield good results after 25 seconds; some negatives need even more than this. If a negative is so thin as to stand no more than five or six seconds, it is too thin to yield really good results unless under exceptional circumstances.

There is no better developer for bromide paper than

the *ferrous oxalate.* We make solutions of neutral potassium oxalate and iron protosulphate, saturated in water at 60 degs. Fahr. To make the iron solution we first acidify the water distinctly with sulphuric acid—about half-dram acid to a pint of water—and then we add the iron salt, shaking or stirring well occasionally. The developer consists of one part of the iron solution to six or eight parts of the oxalate, and to each ounce of this mixture it is advisable to add half a grain of potass. bromide. The separate solutions keep for a long time, but the mixed do not keep long; in mixing the two the *iron should be poured into the oxalate*, and not *vice versâ.*

Note.—The combination of iron sulphate with potass. oxalate produces ferrous oxalate, which is soluble in *excess* of potass. oxalate.

One part of iron to eight of oxalate answers well for the Eastman paper, but if the blacks are inclined to blue one part to six may be used. Thinness of negative may be met by keeping down the exposure and increasing the proportion of iron; over-density may be met by giving long exposure and lowering the proportion of iron; bromide also helps to procure contrast in the print; but an overdose of bromide is apt to lead to ugly greenish tones.

Over-exposure leads to poor images, usually of this greenish tone; under-exposure results in crude chalky images, dense black in the shadows, and undeveloped in the high lights.

The negative is laid face up in a printing frame, the paper face down on the negative, and the frame moved evenly in front of the light. After exposure the paper

is soaked, till it is limp, in pure water; the developer is then poured on, the water having been rejected; the image should not come up for twenty or more seconds; the details should appear one after the other, and density should grow along with the details. When the shadows have taken a good plucky colour—perhaps after three or four minutes—the print is at once to be washed in water acidulated with acetic or hydrochloric acid; if the water smell of acetic acid the acidulation is enough; this is used to ensure elimination of the iron which might be precipitated in the paper and cause a yellow stain. The print is now to be washed well in plain water to eliminate the acid, and is at once fixed in hyposulphite of soda one part, to water five parts, by weight. The development must not be allowed to go too far, for the print appears darker after fixation than before it.

Metol-Hauff may be used as the developer for bromide paper; the solutions of metol and pot. carb. are to be made as directed on page 108, and the exposure must be less than for ferrous oxalate, but the developer must not be diluted more than recommended for negative work, otherwise the tones will be an unpleasant blue or grey. After metol no acid is required; and here again, if the tones are bluish, the exposure has been too great, or the developer is too dilute.

Both the ferrous oxalate and the metol developers may be used repeatedly; in fact, it is a good plan to begin development with a solution that has been used before; if necessary to bring up detail or density a fresh solution may be applied later.

If, after fixing, any marks of a "scummy" nature appear on the prints, an immersion in acid alum solution will remove them quickly; these marks are due to salts in the tap water combining with oxalate in the developer when that developer is used. Metol obviates every kind of staining and marking that we have met with other developers.

The washing must be thorough, as for other prints where hypo is used; the same course may be followed as for gelatino-chloride paper. The squeegee should be freely used; the film of bromide paper is, as a rule, not so tender as that of chloride papers. If a high gloss is desired on the prints when dry the same procedure may be followed as that given for chloride paper on page 122. If the prints are allowed to dry spontaneously on a piece of clean bibulous or ordinary paper the surface will be very pleasing and suitable.

A very high gloss may be given by proceeding thus: Take a piece of good glass, free from surface imperfections, clean it well with spirits and ammonia, or with a paste of whitening and water; then rub it all over with powdered talc—"French chalk"—then rub all the talc apparently off, but do not polish the plate. Next coat the plate with photographic collodion; as soon as the collodion is set, but not dry, wash it under the tap till the greasy appearance, due to the solvents, is gone; then immerse plate and print in water, and bring the two up together, and squeegee them as directed on page 122. When the print is dry, a knife edge may be run round the print, if necessary, and the print will leave the glass

with a very high gloss, which is useful for showing up details in the dark parts of a print.

The Eastman Company have just introduced "enamel bromide paper," "Nikko," which, for our special purpose, surpasses every paper we have seen. It is simply gelatino-bromide emulsion on a glossy and impervious surface. It is used in the same way as ordinary bromide paper, and when washed after fixation it is squeegeed to a waxed sheet of ferrotype plate. It is well to soak the prints after washing and before squeegeeing in a saturated solution of alum for five minutes; this removes danger of the prints sticking to the ferrotype plate. Of course they are to be washed after the alum. When dry, the prints will leave the plate with an incomparable surface, eminently adapted to display the finer details and tones of our subjects.

CHAPTER XV.

Enlarging on Bromide Paper.

It is frequently desirable to produce prints of larger size than the three-inch circle which has been taken as the standard size for negatives; and where mere magnification, and not resolution, is desired, it will be found more convenient in most cases to make an enlarged print from an ordinary negative than to make a negative of unusual size. The process of enlarging is quite simple, and most workers possess all the requisite apparatus; if not, it is by no means costly or complicated.

The original negative requires, in the first place, to be evenly and strongly illuminated; this may be done by daylight from the sky, or diffused by a sheet of ground glass. Or it may be accomplished by an artificial light and a condenser such as is used in an optical lantern; for our three-inch circle, the ordinary lantern condenser of four inches diameter answers well. We next require a photographic lens to project the enlarged image on to our sensitive surface, which must be kept parallel to the negative. And lastly, we require to arrange so that no light reaches the sensitive surface except what passes through the lens.

The simplest apparatus for our purpose consists of two cameras fixed together—one small to take our small negative, the other large enough to hold our largest

sheet of paper. The small camera may be of the simplest kind. It requires no "movement" except a focussing one; in fact, a "toy" camera will do. The large camera requires to be adjustable to various lengths, and it is convenient to have it with a certain amount of vertical and horizontal movements. Fig. 26 shows our apparatus made up of two such cameras, the larger taking a plate or sheet 10 by 8 inches. The lens—of about $3\frac{1}{2}$ equivalent focus—is fixed to the small camera, and the dark slide of the latter is used to hold the negative. The

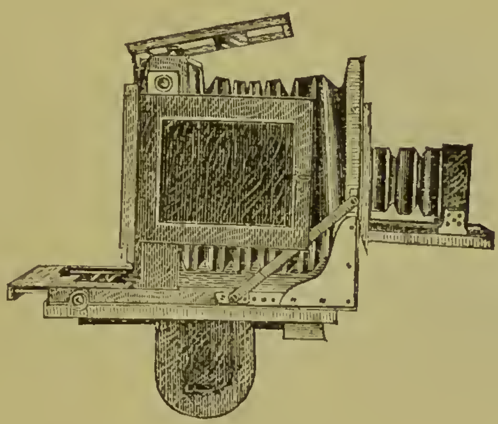


FIG. 26.

sheet of bromide paper is placed in the dark slide of the large camera, being backed by a sheet of glass to keep it in position; and the whole apparatus is either turned towards the sky, or towards a piece of ground glass in a window. If the small camera is turned towards the sky, and if there are clouds, or trees, or anything coming in the field and in the focus of the lens, a piece of ground glass must be put about two inches behind the negative.

The amount of enlargement is regulated by the distance of the lens from the small plate, and the focus is adjusted on the ground glass of the large camera, or on a piece

of plain glass placed *pro tempore* in the dark slide, the paper being put in when the focus is adjusted. If the image does not fall on the centre of the paper this is corrected by moving the rising or traversing front of the larger camera carrying with it the small camera. Of course it is necessary that the junction of the two cameras shall be light-tight. A piece of velvet will effect this in the simplest manner.

If we only wish to enlarge to such size as can be taken by our photo-micrographic camera, we have at

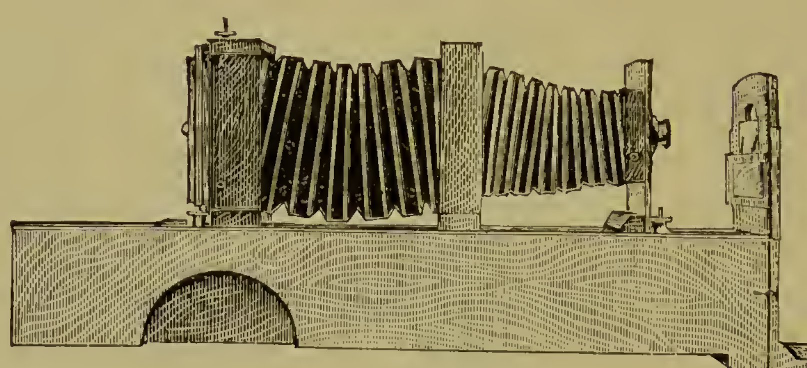


FIG. 27.

command an apparatus even simpler. In fig. 27 we show our photo-micro camera, its support carrying an easel for the small negative. This easel has a certain amount of vertical and horizontal motions, and will carry, if required, a quarter-plate negative. The negative is simply illuminated by diffused daylight, and "copied," but the lens is placed at such a distance from the negative and from the sensitive surface that the size of the image is about doubled—in diameters—on the paper. In place of the safety cap on the front of the camera, used in photo-micrography, a lens flange is adapted taking our short-

focus photographic lens. The accurate focus is got by sliding the back of the camera in the usual way. This apparatus is of great value in making negatives of such objects as are too large for a microscopical lens; we often use it for large sections which are to be enlarged only a few diameters. Till we adopted this device these large sections were a source of great trouble to us.

For enlargement by artificial light we use what is practically an optical or "magic" lantern, with a four-inch condenser, and we project the image upon a sensitive surface—bromide paper, in place of the ordinary screen. But as the distance between lens and screen is not great, it is better to have the lantern and screen or easel fitted to a base-board; the easel should slide along this base, and should have up and down movement The negative is placed in a "carrier" as usual, in the lantern, and the side movement is obtained by the use of this carrier. The operation is preferably carried on in the dark room, for this obviates the necessity for light-tight arrangements between lantern and sensitive paper; but the lantern should either be placed in a box or so fitted that no light escapes from it to fog the paper. The front of the lantern should have a stretch at least half as long again as the focal length of the lens, and a lens of moderately short focus should be used, say 3½ or 4 inches. The negative must be evenly lighted, and if any difficulty is found in arranging this, a piece of ground glass may be put inside the lantern between light and condenser; but this necessity should not arise. The "Cantilever" apparatus of Hume, of Edinburgh, is a good example of enlarging apparatus,

but a short-focus lens should be fitted to it (fig. 28). To give an idea of the stretches that may be required, we may state that, with a lens of 4 inches equivalent focus, to enlarge two diameters we require $\frac{12}{6}$, three diameters $\frac{16}{5 \cdot 33}$, four diameters $\frac{20}{5}$ inches, the numerator giving distance from lens-centre to sensitive surface, the denominator the distance from lens-centre to negative.

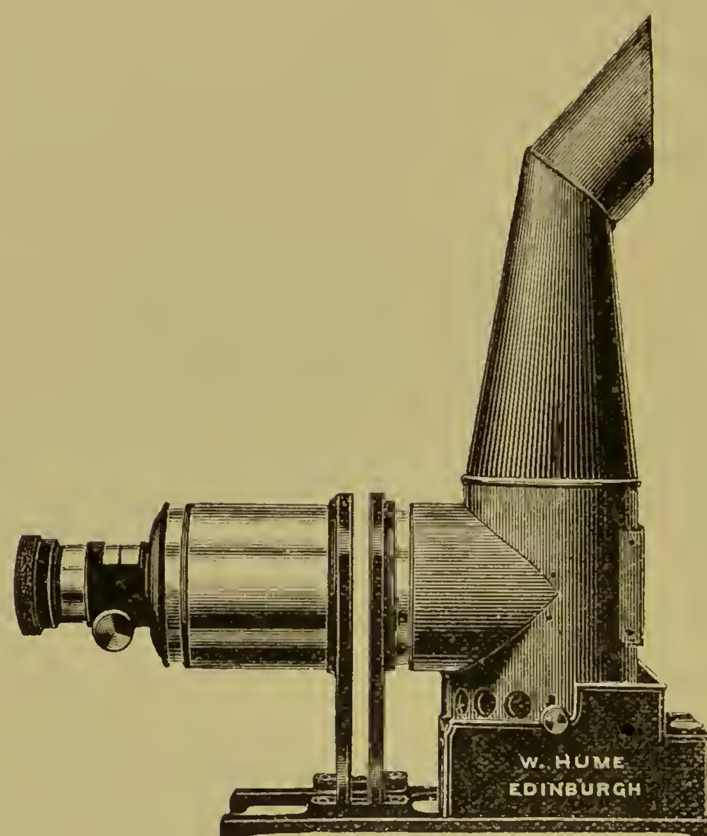


FIG. 28.

Exposure follows here the same rules as for contact-printing on bromide paper; but, if desired, a more rapid paper may be used. The exposure should be so regulated that the prints develop slowly, free from fog and from greenness. It is impossible to lay down the time of exposure, or even to give useful hints, as all depends on

the light, the negative, and the emulsion. When the condenser is used in the lantern the exposure required will be very short, unless the lens is well stopped down. It is to be noted that the diaphragms of photographic lenses are usually so arranged that each one necessitates double the exposure required with the next larger diaphragm. There is very little gain, in enlarging, by using small stops, but sometimes the exposure with the largest stops is inconveniently short; this is often found with a condenser and extra-rapid bromide paper.

The operations of development, fixing, washing, etc., are the same as given in Chapter XIV.

As illuminant, in enlarging with a condenser, we may use an oil lamp or limelight. A friend of the writer produces excellent work in this way with two Welsbach gas burners in a Cantilever lantern, the burners being not quite in a straight line one behind the other.

CHAPTER XVI.

Lantern Slides.

OF all the forms under which we can show the results of photo-micrographic work, none is so useful or so striking as a good "slide" well projected on a good screen. For purposes of demonstration and education, no kind of illustration can approach this; the size of the image is great, the details clearly shown. Many persons can observe the picture at once, and the demonstrator can direct special attention to any detail. Moreover, the slides themselves are usually better "prints" than any on paper, and in almost all cases a lantern slide viewed in the hand gives a better representation ot an object than can be obtained by any process of printing on paper, the transmitted picture being, in fact, superior to the reflected. And, happily, the process of producing slides is by no means difficult to learn, the chief desideratum being to know exactly what is required, and to know a good slide when we see one.

The first condition of a perfect slide, specially of our class of subjects, is perfect *clearness* of the ground; where there is no image the glass of the slide must be quite clear. Next, there must be transparency in the shadows, *i.e.*, in the image of the object; if the image-details are blocked up by over-density or fog, the slide cannot be good. Again, the whole must neither be too dense nor black-and-white, nor yet pale and "washed-out" in appearance; we require *pluck* without *hardness*. Lastly, the *colour* of the

image must be suitable; and for our purpose this colour should be a good black, or as nearly approaching black as possible. Happily, this tone is the easiest of all to get with such slide-plates as we shall recommend; it is difficult to obtain warm tones without inducing fog.

We do not propose to run the risk of confusing the reader by discussing more than two kinds of plates; and it happens that the same developing solution answers well for both. Moreover, as probably the worker has made his negatives on quarter-plates, or on lantern-slide-size plates, we need give but little attention to any process of printing except "by contact."

Presuming that the negative is nearly the size required for a lantern slide (a plate $3\frac{1}{4}$ inches square, with probably a $2\frac{3}{4}$ or 3 inch picture on it), the printing is performed as if the slide-plate were a piece of paper; the negative is laid face up in a printing-frame, the slide-plate face down on the negative; the frame closed, and the whole exposed to light as in the case of a print on bromide paper. But there is a slight advantage in copying the negative in the camera, as thereby a somewhat greater sharpness of definition can be obtained; the advantage, however, is probably not balanced by the extra trouble to most workers. But if we have to make a slide from a negative larger than three inches we must reduce it. This is effected in a camera similar to our enlarging one, but turned the opposite way as to the light; the larger plate is turned towards the light. The small camera contains a slide-plate, and the negative is simply copied on a smaller scale on the slide-plate.

Slide-making on Gelatino-chloride Plates.

These plates ought to be of the slow kind ; some chloride plates are sold as "rapid," being intended for use in the camera, but as a rule they are distinctly inferior for our purpose to slow chloride plates. These plates are only slightly sensitive to yellow light, such as gas or candle, and the exposure should be made to daylight or magnesium ribbon. Two or three seconds to daylight diffused by ground glass will be found suitable for an average negative; or two or three inches of magnesium ribbon at a distance of a foot may be more convenient and equable. The proper exposure must be learned by experiment; once known for a few negatives it will be easy to judge it for others. The ferrous oxalate developer given on page 126, will answer very well for these plates; but a small dose of common salt—say one grain to each ounce of developer—may be used as a restrainer. The following developer was given some years ago by Mr. A. Cowan. The exposure is to be kept down, and the tone obtained will be cold, a good blue-black.

A.	Iron protosulphate	140 grains.
	Sulphuric acid	1 minim.
	Water	1 ounce.
B.	Potass citrate	136 grains.
	Potass oxalate	44 ,,
	Hot water	1 ounce.

The developer consists of 1 part of A. to 3 parts of B.

With chloride plates the image usually comes up rapidly, and gains density slowly; time must be allowed for the acquisition of density, as much of it is apt to disappear on fixing. The plates are fixed in hyposulphite

of soda, one part to five of water. It is a good plan to develop a plate to considerable density, and, if necessary after fixation, to reduce the density with the ferricyanide reducing agent given on pages 115-116, or with the following, due to Mr. Cowan :—

Solution of iron perchloride, B.P. ...	$\frac{1}{4}$ ounce.
Hydrochloric acid	$\frac{1}{2}$,,
Water to	20 ounces.

Soak for a short time in this, wash, and then immerse in fresh hypo solution. If any scum is found on these plates after any of these processes, it may be at once removed by the acid alum solution (page 111).

Gelatino-bromide Slide Plates.

These are the plates generally used for slide-making, the emulsion on them being practically of the same nature as that on bromide paper. The less sensitive the plates are for our purpose the more likely are we to succeed with them. We do not hesitate to recommend for trial the "black tone" plates of Messrs. Cadett and Neall, and good results may also be got with those of Messrs. Thomas and Co. Even with the slowest gelatino-bromide slide plates obtainable reductions may be made in the camera with a lens at *f*/16 by a few seconds' exposure to diffuse daylight; and the exposure to a fishtail gas-burner at 12 inches distance, "by contact," is from 10 seconds upwards.

No developer answers our special purpose so well as the *ferrous oxalate*, the solutions of iron sulphate and potass. oxalate being kept as directed on page 126, and mixed in the proportions of one part of iron to six parts of

oxalate, with half-a-grain of bromide to each ounce of developer. The process of development is very similar to that with bromide paper, but we must carry the density to a higher point, as much is lost in fixation, and we require a pluckier image. The washing, in acidulated water, after development, must not be omitted; and, if after fixing a scum appears on the film, it is to be removed by pouring on and off the acid alum solution.

Sat. solution of alum in water	10 ounces.
,, ,, of citric acid in water	10 ,,
Hydrochloric acid	2 drams.

If any tendency to frill the film is found in the use of this solution, the quantity of HCl may be reduced.

As usual after fixation in hypo, the plates must be thoroughly washed in running water or under the tap. Very neat porcelain troughs with grooves for the plates are to be found on the market; each trough holds a dozen plates, quarter-plate or slide size, and the water is run into the trough for an hour or two. And small glass dishes are sold for lantern-slide plates; but when the exposure is learned several plates may very well be developed in one large dish, for the development should not be very rapid. If a bromide plate develops with ferrous oxalate in less than three or four minutes it will probably be found to have a greenish tone, which is a *sure sign of over-exposure.*

The *Metol-Hauff Developer* may be used for this work; the solution as formulated on page 108 must not be diluted, and exposure must be kept down, but bromide may be added to the developer without impairing the tone of the resulting slide.

The blacks obtained with metol are of a somewhat warmer character than with ferrous oxalate. Another developer well worthy of trial, because it seems almost incapable of fogging a plate, is the *Glycin* of Messrs. Hauff. It may be made up as follows:—

Glycin-Hauff	5 parts or 75 grains.	
Potass carbonate	20 ,, or 300 ,,	
Sodium sulphite	20 ,, 300 ,,	
Water to	100 ,, or 3 ounces.	

For use dilute one part of this to three parts with water. This developer ought to act very slowly if the exposure has been correct ; ten minutes will not be too long. Here, too, bromide may be used if the development is too rapid, or the high lights show tendency to fog. But if the exposure has been nearly correct, no bromide will be required, or a very small quantity at the most.

A poor lantern slide may almost always be attributed to a poor negative, for it is easy to make a good slide from a good negative. But sometimes it is impossible to obtain a really plucky negative, the nature of some objects precluding this; in such a case, where the negative is wanting in contrast, the best plan is to keep the exposure as short as is compatible with getting the details developed, to develop very deeply, and after fixation, if necessary, to reduce with weak ferricyanide, carefully watching the progress all the time, and stopping it when the lights are clear and the image as little affected by the reducer as the process will allow. When we have a very dense black-and-white negative, the plan is to give an exposure which appears even in excess of what

would be required to give the details, and to develop no further than is necessary to yield the required density.

Whatever developer is used it will always be found advantageous to use the clearing solution of acid alum; the effect is not invariably patent to the eye, but we believe the step is a wise one. And varnishing the slide has always had a beneficial effect, not only in tending to preserve the slide from damp and mechanical injury, but also in clearing it up. When the plate has been dried, a colourless varnish, usually cold, is flowed over it; and a varnish lately introduced by Messrs. England Bros. appears to us as good and as convenient as any. This varnish is applied after the manner of collodion, being poured in a small pool near one corner of the plate, and caused to run evenly all over the film and off at the opposite corner back into the bottle whence it came; it is important to have the varnish filtered after each time of use, for any dust on a slide appears as a large blemish on the screen. When the varnish has dried, a short time at ordinary temperature, the slide is ready to be "mounted."

The mounting of a slide is for its preservation, and the process consists of laying on the film a "mask" of opaque paper, having a suitable aperture cut out of it; laying over the mask a sheet of glass 3¼ inches square; and binding the edges of the glass plates with adhesive paper "strips." It is very convenient to have the masks black on one side and white on the other; in mounting, the white side is laid uppermost, and the title of the subject written on it, with any other particulars desired. It is also well to have masks with various sizes of apertures; frequently we do

not wish to show as much subject as occupies a three-inch circle. Wormald's masks and strips are worthy of recommendation. If it is of importance to show the image on the screen with a particular margin upwards, the mounted slide is laid down on the table as it is wished to appear on the screen, and two small marks are put on the cover-glass at the *two top corners* of the slide; this is a standard mark for the lanternist. In the lantern the two marks go into the carrier next the light and downwards.

Mention might have been made of other processes for slide-production, as wet collodion, which is almost unapproachable for the purpose; albumen, and dry collodion. Other developers might also have been given, as hydrokinone, pyrogallol, etc., but for the reason given in the early part of this chapter we prefer to omit these; details of them will be found in photographic literature.

CHAPTER XVII.

Microscopical Technique. Cover-glass Preparations. Section-cutting and Staining.

FOR the production of perfect photo-micrographs, two things are required—good preparations and good photography. We have tried in the foregoing chapters to make clear the way to good photography, but as an imperfect preparation will never yield a perfect photograph we propose to devote this chapter to an outline—it can be no more—of the methods we have found the best for the production of good preparations. These suggestions must be more or less confined to medical microscopical technique, simply because the writer has no trustworthy knowledge or experience of any other. The preparation of insects, diatoms, botanical specimens, etc., are no less important, but while the general principles involved are the same as for medical work, we elect to give no suggestions on these subjects rather than perfunctory ones which might be misleading.

Whatever system of staining we employ, the stain must be *discrete* and *selective*. So far as possible, each kind of tissue should have its own stain, and the stains must not run beyond the tissues, nor into each other. All dyes should be avoided which stain many tissues indiscriminately—"diffuse" stains; and we should employ stains having special affinities for the tissues or bodies to be

examined and photographed, so that we shall either get none but the bodies or tissues under investigation stained, or have the whole preparation deeply stained first, and the stain afterwards removed from all but the special bodies or tissues,—*Maximum Decolourisation.*

The point is to get one tissue visually and photographically distinct from others. Acid haematoxylin will hardly stain anything but nuclear tissue; benzo-purpurin will stain indiscriminately the whole of the tissues in a section if time is allowed, and unless we take steps to prevent this diffuse action. And some of the basic aniline dyes will stain bacteria, nuclei, tissue and all, but they are removed from all except the bacteria by suitable—and, in our case, necessary—procedure. Moreover, these anilins, if improperly used, will stain bacteria in such a way that the outlines of the organisms will be "fuzzy"—in fact, the staining action will extend beyond the bacteria, and give them unsharp outlines. Again, by neglect of proper "fixation" of a tissue, we may get its elements disintegrated, and unrecognisable, and false in appearance. Lastly, in the processes of preparation, we may actually damage and destroy our objects; as when, by over-heating a cover-glass preparation, we "frizzle" it into a totally misleading shape.

Photography rightly prides itself on its accuracy, and if, in our precedure preliminary to the photography, we alter the appearance of our objects, then our photography simply accentuates and perpetuates a falsity. It is true that every method of preparing tissues for microscopic observation alters more or less the appearance of the object; but

it is in practice necessary to prepare our tissues in some way and to some extent; our duty, then, is to alter them as little as possible. This duty is kept firmly before our eyes in the necessarily brief remarks that follow.

The usual plan for fixing substances, *liquid or semi-liquid*, spread on the cover-glass—to prevent the substance from being washed off in later operations—is to heat the glass to such an extent as to coagulate the albumen; and doubtless this is effective and safe in careful and competent hands. But many a preparation has been spoiled by this treatment; and an equally effective and much safer plan is to immerse the cover-glass for even a few seconds in saturated mercury perchloride. We have never known a substance containing any albumen washed off after this treatment, and the sublimate acts as a mordant for the stains that usually follow. (If the substance contains no albuminous matter the heating will not fix it to the glass).

In almost all cases it will be found best to immerse *solid* tissues, as soon as possible after removal, in a similar solution of sublimate; sodium chloride or acetic acid may be added to the water for solution, but we find no need for it; and it seems to make the future elimination of the sublimate more difficult. Granted that, for the central nervous system, and where it is important to preserve blood in its relation to other tissues (Woodhead), Müller's fluid may be preferable; and for certain purposes Klein's fluid (chromic acid and spirits) or Foà's (sublimate and potass. bichromate) may be useful or necessary; still, for general purposes, nothing is probably so good as saturated sublimate. The pieces of tissue

are to soak in this from 12 to 24 hours, according to their size. They are then washed from 8 to 12 hours in running water, or in many changes of dilute spirits not under 35 per cent. For washing in water, a china tea-pot may be used; the lid-hole covered with muslin, the water run into the spout briskly from the tap. The strength of the spirit, after washing, is gradually increased up to 70 per cent.; and in this the tissues are kept till the actual preparations for section-cutting are begun. In preparing tissues for physiological or pathological investigation we usually have two ends in view: first, to study the tissues in general or their relations to each other and to surrounding tissues; and, second, their intimate characters, or histology. Thus, in pathology, we wish to know the relation of the diseased parts to the surrounding healthy tissues, and we also wish to discover the histological facts of the diseased tissue. For the first purpose it is necessary to take a bit of the abnormal part where it abuts on the normal, and this bit should be as large as can be conveniently manipulated. For the second purpose we need not take so large a portion, provided we are sure of taking a typical part of the disease. This second part must be cut as thin as possible; the first part need not be so thin, but should certainly not be thick. A moderately-thin section may be 5μ (about $\frac{1}{5000}$th inch) thick, a really thin section is one about 3μ ($\frac{1}{8000}$th); a section over 8μ ($\frac{1}{3000}$th) may be called thick.

In our opinion and experience, it is not possible to depend on getting really thin sections by any process except the *paraffin*; and, further, there is no process like

the paraffin for preserving the relations of the component tissues to each other when the sections come to be cut. With frozen gum thin sections may be cut, but the sections are apt to be torn and folded in after-operations, no matter how skilfully executed. With celloidin really thin sections can seldom be cut; moreover, the celloidin is apt to take some of our stains, which cannot easily be removed from the celloidin, and a confused "picture" is produced. With paraffin infiltration the thinnest sections can be cut; the true histology is fairly well preserved; the relation of one tissue-element to another is preserved excellently; and the paraffin can be removed from the sections without the risk of loosening the tissue from its support, before the staining operations are begun. We therefore strongly recommend the paraffin-infiltration process to photo-micrographers—and, for that matter, to all who wish to study physiology, normal or abnormal, under the best conditions. The paraffin process does not take longer time than the gum, for tissues to be cut in gum require to be more hardened than those to be cut in paraffin, according to our experience. If we have a tissue properly prepared for embedding in paraffin, we look upon it as a certainty that we shall get good sections.

The tissue, supposed to be in 70 per cent. alcohol, is soaked for 24 hours in "neat" methylated spirits, then for two periods of 24 hours each in fresh absolute alcohol. Then with a pipette methylated chloroform is put under the alcohol, and the tissue is left there till it sinks down into the chloroform, or for 24 hours. It is next soaked for 24 hours in chloroform, the tube containing it being

kept in a warm place and loosely stoppered. Mineral naphtha may be used in place of, and in the same way as, chloroform. We prefer these volatile substances to cedar oil and the like, in view of the exhaustion process which is to follow; but if this process is not to be performed, cedar oil is the best paraffin-solvent to use. We place the oil at the bottom of the tube, on it we pour carefully sufficient absolute alcohol to cover the piece of tissue, and on the alcohol we carefully place the tissue taken out of the second dose of absolute alcohol. When the tissue has sunk down into the oil it is put into oil alone, and left till it is clear, the oil having then fully impregnated it.

After the chloroform, the naphtha, or the oil, the tissue is placed straight into melted paraffin; we can discover no use for intermediate baths of paraffin in a solvent. If oil has been used the paraffin is changed after some hours for fresh; after treatment with the volatile substances we strongly recommend the use of an air-pump, or a water-filter pump. When the tissue is thoroughly warmed in the paraffin, the vessel containing it in the paraffin is placed under the receiver of an air-pump, and the air exhausted; bubbles will at once rise, and the exhaustion is to be kept up till no more bubbles rise; the tissue is then put into fresh paraffin. The same course is followed with the water-pump; the tissue is in a tube of glass, and this tube is attached to the pump, and so left till no bubbles appear. Of course, the paraffin must be kept melted by the application of heat; but for a full account of these matters the reader is referred to the *Journal of Pathology and Bacteriology*,

Vol. I., No. 1, May 1892. [Pentland, Edinburgh.] This system of exhaustion greatly accelerates the process of infiltration, but its chief advantage lies in the facts that it makes the penetration of the paraffin more certain and more complete, and that one can tell when the process is complete by the cessation of the bubbling.

With regard to the foregoing, it may be noted that to ensure the dehydrating alcohols penetrating the tissues, it is well either to suspend the tissues in muslin bags in the alcohols, or to place at the bottom of the tubes small plugs of lightly-packed cotton-wool. If any failure occurs in the paraffin process it will almost certainly be due to imperfect dehydration. Some tissues, as lung, float on the alcohol and paraffin solvent; if this is due to air in the tissue the latter may be pushed down into the liquids, and held there by a plug of wool; if due to the specific gravity of the tissues, ether may be added to the chloroform till the tissue sinks. It is important to adjust the melting-point of the paraffin to the temperature at which the sections are to be cut. In winter, or for a cold room, the melting-point may be about 46° to 48° C.; in summer, or a warm room, 52° or even 56° C. may be advisable. This is a matter for experiment and of experience; no rule can be laid down. If the paraffin is too soft, the sections will be folded and wrinkled when cut; if too hard, they will come off the knife in rolls like tiny pencils. We have never known tissues spoiled by the heat needed for paraffin processes if the fixation and hardening of the tissue had been properly carried out. But the principle to follow is to use as soft paraffin as will allow good sections to be cut,

or to make the room where they are cut as cold, within limits, as possible. By far the most convenient and trustworthy stoves, or incubators, known to us are those of Hearson. We have used several for years, and have never had the slightest hitch with them. When not in use they are turned out, and no readjustment is required when they are relighted—this touches the gas stoves only; we have no experience of the others. The paraffin stove should run about 4 or 5 degs. Fahr. above the *m.p.* of the paraffin. The impregnation by paraffin may be conducted in small dishes known as "Cheese-Ramequin dishes," and in this case the tissues remain in the dish till impregnation is complete; the dish is then floated on cold water till a thin skin of cooled paraffin forms on the top, when the dish is pushed under the water, and the whole mass of paraffin cooled *as rapidly as possible.* The dish is then placed in hot water, and the mass removed, and trimmed for the microtome. The metal L pieces may also be used.

The simplest microtome made is that known as the *Cathcart*, and when this is used with a plane-iron set in a wooden handle, wonderfully good sections can be cut with it. It is, however, hardly the scientific and accurate instrument required for the best work, and an instrument of higher class will be found in the *Cambridge rocking microtome.* This, however, cuts sections which are really segments of a circle, which is not quite conducive to facility in after treatment. At the same time, very fine sections are cut with it, provided we do not attempt too large pieces of tissue. The instrument which we consider the best is the *Minot* (fig. 29), which may be obtained

from Mr. R. Kanthack, London, and this we say after considerable experience of several of the best-known microtomes. This instrument holds the tissue firmly, and cuts it in straight lines, and the devices for "orientation," and for regulating the thickness of the sections, are admirable. In the simpler form the

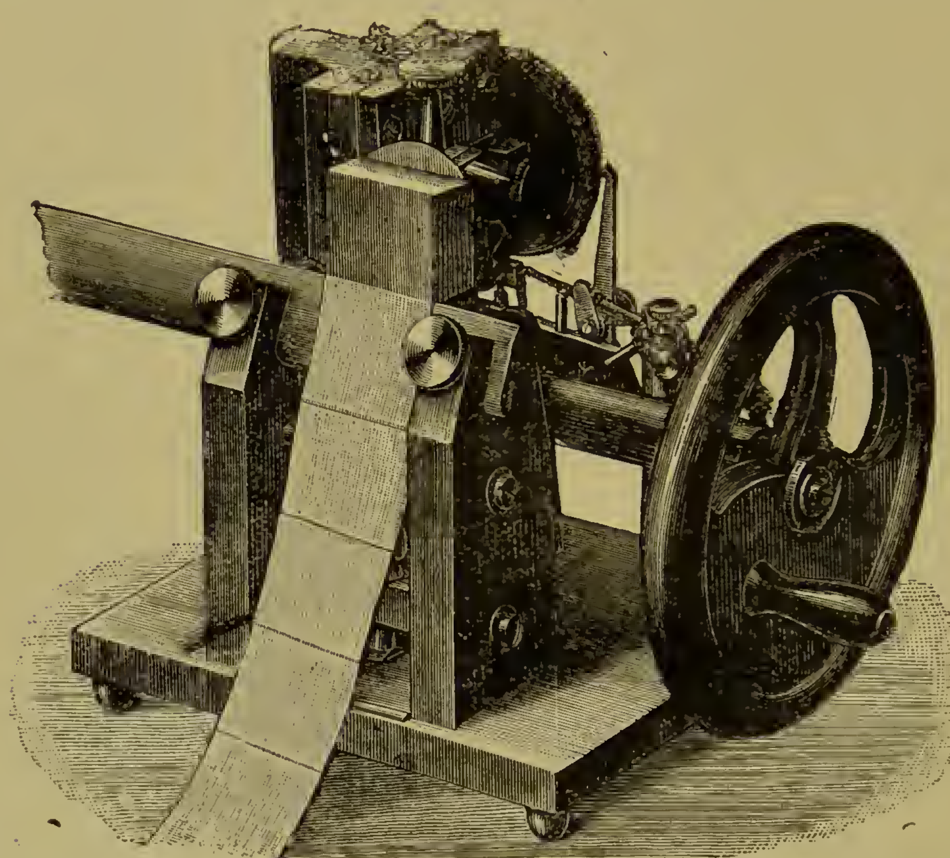


Fig. 29.

thinnest section that can be cut is the 300th of a millimetre, and this comes well within our definition of a thin section; but another form is made, which will cut sections considerably thinner. Another point is that with this microtome we can section tissues with a surface of an inch and a half at least. For surfaces larger than this, recourse must be had to the *Thoma* or the *Schanze*,

or, better, to such an instrument as has lately been designed by our friend Mr. G. L. Cheatle, of King's College.

The sections having been cut either in chains, or separately, are laid on clean glass slips, and filtered water is run in beneath them with a pipette, so that the sections float on the water. Heat is now applied to the lower side of the slip till the water is of such heat as *almost* to cause the paraffin to melt. The sections under this treatment will open up in a most satisfactory way, and this spreading may even be aided by a camel-hair brush. It is astonishing to what an extent we can open up an apparently fatally folded section in this way. The water is now run off the slip, and the slip with its section is allowed to dry naturally or in the cool incubator, about 100° Fahr.

When the sections are *quite dry*, the paraffin is removed by two baths for a minute each in mineral naphtha, and the latter is removed by a bath of spirits. (The dry slips and sections may be kept for any length of time before the paraffin is removed.)

The foregoing processes are in the main due to Dr. Brook and Mr. Gulland, of Edinburgh. Sections treated as above will hardly leave the slips under any provocation; but sometimes tissues which have been thoroughly tanned with "Müller" or a chrome salt will leave the slips. In such cases we must use the fixative solution of Mayer—albumen and glycerine. But the former process is far superior when it is effective.

This is not the place for us to go at great length into staining processes; nor is it necessary, for sections *well*

stained by any process will yield good photographs. The well-known picro-carmine, for example, is excellent for photo-micrographic purposes, but it will not be amiss to describe what seems the best process for our special work, leaving out notice of special microscopic researches, such as those into cancer-parasites, specific chemical reactions, etc.

Ehrlich's Acid Hæmatoxylin.—Hæmatoxylin 1½ gramme dissolved in absolute alcohol 100 c.c., add glycerine and distilled water each 100 c.c., glacial acetic acid 10 c.c., and potash alum to excess. Allow to stand in a light place for some weeks. The older this is the better, but it must be red and not purple in colour. If it becomes purple add acetic acid. This should stain nuclei in about five minutes, or ten at the most. If in place of hæmatoxylin *hæmatein* is used, the solution will be ready for use much sooner; and if this hæmatein is diluted to such an extent that it stains thin sections in about 18 to 24 hours we shall have perhaps the most exquisite nuclear stain in existence. If it is not intended to counter-stain, or if absolute discreteness is not necessary, the preparations are washed in tap water directly from the stain; but if we propose to stain other elements besides nuclei, the sections must be washed in *distilled* or acidulated water, and then in tap water till they take a true blue colour. If the tap water is not distinctly alkaline, a little alkali may be added to it; the alkali *fixes* the stain in the tissues, and gives it the blue colour which is a sure sign of the fixation.

The next step is to "counter-stain" the section, though

this is a term not wholly applicable; for what we do is to stain, equally discretely, other tissues with other dyes. Make saturated aqueous solutions of "acid rubin" and "orange" (Grübler), and mix them in the proportions of two of orange to one of rubin, and then dilute the mixture with water till it stains sections dark red in about five minutes. After the sections are stained in this, wash in water till they lose no more colour in the water, then wash in spirit, then dehydrate, clear in mineral naphtha, and mount in xylol or benzole balsam. Instead of the naphtha we may use Weigert's solution, one part absolute phenol to three parts xylol, but clove oil should be avoided for every tissue, every stain, and every purpose. Now it frequently happens that the orange which is intended to stain "protoplasm" is all washed out by these operations, and search was made for some protoplasmic stain which would not wash out so readily. We finally adopted *benzo-purpurin* (Squire). A "stock" solution is made as follows:—

Benzo-purpurin	1	part.
Alcohol	20	parts.
Water to	100	,,

To make the stain for use, take of the stock benzo-purpurin 10 parts, of the saturated rubin solution 4 parts, and make up with water to 100 parts. The rubin will stain the connective tissues, and the benzo-purpurin the protoplasm, and the latter stain will not wash out in water or spirits to any great degree. There is in some minds a suspicion of the permanency of this stain; but at any rate it keeps well in balsam for more than a year.

This dye, benzo-purpurin, stains cell-protoplasm so well

that we have used it for leprous tissue, to study the relation of the "rods" to the cells, in this way: The bacilli were stained in the usual way with carbolic fuchsin, the tissue decolourised in mineral acid, the nuclei stained with acid hæmatoxylin, and the cells with very dilute benzo-purpurin, the result being extremely instructive and useful. Such subjects as require special staining for their demonstration must, of course, be treated as their nature demands; but this may be said, if sections are well cut and the staining process a good one, and well carried out, there will be little or no difficulty in photographing the preparations. With Weigert's stains for the central nervous system, and with any of the well-known modifications of that method, we have found no trouble in getting good photo-micrographs when the sections have been reasonably thin. So, too, with the Ehrlich-Biondi, so much used at present; and so with all the variations of the carmine stains.

For *bacteriological preparations* the point, as already stated, is to have the organisms discretely stained and well washed; but it is also necessary with the blue and violet dyes to have the stain *dark*. Gentian—or even methyl-violet—photographs beautifully when the organisms are deeply stained; the same holds good with methyl-blue, but if these stains are only pale, and particularly if the sections are counter-stained, the trouble in getting a good plucky negative showing the relations of organisms to tissue is infinite. Methyl-blue is a special sinner in this respect, that it washes out very easily, and some clearing agents too often used, as clove oil, are apt to destroy it for photographic purposes. We much prefer, when

it is possible, to stain the bacteria red, with fuchsin or dark violet, by Gram's system, and to stain the tissue blue as with the "plasmamethylene-blue," or red with alkaline carmine, or yellow with benzo-purpurin, as the case may be. The bacteria, as seen in the microscope, must stand out boldly from the tissue; if this is the case it is our business to overcome any colour-difficulty there may be. But if the bacteria do not stand out, it cannot be expected that we shall make them appear to do so in our photographs. For a good photo-micrograph, we repeat, we require a good preparation and photographic skill.

The faintly alkaline methylene-blue of Loeffler stains cover-glass preparations and bacteria in sections beautifully, provided the preparations are not over washed. The same may be said of the carbolic blue (methyl) of Kühne and others. Weigert's method of making stains also answers well—anilin water—but we add alcohol in excess of the usual quantity. The addition of alcohol certainly tends to make the staining more discrete. When fuchsin—carbolic or anilin—is used, the stain need not be so dark, and for various reasons the writer uses fuchsin to stain bacteria when its use is permissible. But if this stain is too heavy, much detail of the organisms may be lost in photography.

Tubercular tissues and sputum preparations show extremely well when treated as in the cases cited on page 155 for leprous tissue.

APPENDIX.

COST.

FOR the convenience of the reader we give here a list of approximate prices of pieces of apparatus mentioned in this book, and a few not mentioned in the text. It is, however, to be understood that prices being subject to variation, neither manufacturers nor dealers are to be bound by the prices given.

STANDS.

	£	s.	d.
Powell and Lealand, No. 1, with 2 oculars	42	0	0
,, ,, No. 2, ,, ,, ...	28	0	0
,, ,, Students'	11	11	0
Baker's "Nelson," fig. 1 ... ... about	12	0	0
,, ,, as in fig. 8.	30	0	0
Watson's "Edinburgh," fig. 4, 1 ocular	9	10	0
Swift's Histological, fig. 5A, 1 ocular	6	15	0
Beck's Biological, fig. 5B, 1 ocular, 2 objectives, with case	9	10	0

CAMERAS FOR PHOTO-MICROGRAPHY.

With focus arrangements, etc.. . ... from about	5	0	0
The Apparatus, fig. 7, complete with microscope	100	0	0
The Apparatus, figs. 8 and 9, without microscope about	8	10	0
The Van Heurck Vertical Apparatus, without microscope, fig. 10	4	15	0
Author's Vertical Apparatus, fig. 11, etc., without microscope, about	10	0	0

CONDENSERS.

Zeiss Achromatic, N.A. 1, fig. 13	3	15	0
Abbe Illuminator, N.A. 1·2	2	2	0
Powell and Lealand Achromatic, N.A. 1, with fittings, fig. 14	8	8	0
,, ,, ,, N.A. 1·4, ,, ,,	15	15	0
An Ordinary Achromatic Condenser with stops, about 160°	3	3	0
Paralleliser, Nelson's, fig. 21 ... ... about	1	1	0

OBJECTIVES.

The most costly are the apochromatics of Zeiss; next come the apochromatics of Powell and Lealand, and other makers; next, the "quasi-apochromatics" of various makers, as Leitz, etc.; next come the better-class students' glasses, *e.g.*, oil-immersion objectives of high power and fair aperture; twelfths, by Swift, Beck, and others; N.A. about 1 : 3; prices about £8 to £10; lastly, low-angled objectives, achromatic.

Powers being equal, prices vary with apertures, a large aperture glass costing more than a narrow aperture of the same focal length.

ILLUMINATING APPARATUS.

	£	s.	d.
Oil Lamp, large wick, fig. 19	3	0	0
Swift's Nelson-Dallinger Lamp, with double paralleliser, small wick	2	10	0
,, ,, ,, ,, Iris, extra	0	12	0
Heliostat, fig. 18	5	10	0
Newton's Lime Jet, "Pringle Cut-off," fig. 20	2	7	6

MISCELLANEOUS.

	£	s.	d.
Enlarging Apparatus, Hume's Cantilever, 4in. condenser, projection lens, etc., fig. 28	3	15	0
Microtome, Minot	10	0	0
,, ,, with knives and sundry fittings... ...	13	0	0
Paraffin Stove, or Incubator, Hearson's patent, 12 by 12 by 14 inches inside	6	10	0

The purely photographic outfit—dishes, lamp, chemicals, etc.—ought not to cost more than £1 10s. or £2.

These prices represent the *best* articles of their kind. In many cases much cheaper instruments, if well chosen, would suit for good work.

ERRATA.

Page 96, line 3—for *Appendix* read *last chapter.*
Page 103, line 10—for *plain* read *plane.*